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BULLETIN NO. 149

TUBERCULOSIS OF FARM ANIMALS

By CHAS. F. BRISCOE and W. J. MacNEAL

URBANA, ILLINOIS, FEBRUARY, 1911

Summary of Bulletin No. 149

1. Tuberculosis was known as early as 460 B. C. It is a disease due to a specific organism, *B. tuberculosis*. Four types of this organism are recognized; the human, bovine, avian, and a type belonging to cold blooded animals.

2. The disease is characterized by the formation of tubercles as a reaction of the animal tissues. The tubercle bacilli may enter the body thru the act of breathing, eating and thru wounds; and leave the body thru the mouth or nose, or in the milk, feces, urine, genital discharges, or discharges from wounds. The tuberculous animal rarely shows physical signs until the last stages of the disease.

3. In determining the presence or absence of tubercle bacilli in material supposed to be infectious, the method of animal inoculation is far superior to that of microscopical examination. This is true because of the difficulty of distinguishing the tubercle bacilli from other acid-fast bacilli, and the difficulty of finding them when present in small numbers. Pages 317-327

4. Tubercle bacilli are widely distributed in market butter. Table 1 shows a total of 1233 samples of butter tested of which 163, or 13.2 percent, were positive; Table 2 shows 209 samples of oleomargarine tested, with 9, or 4.3 percent, positive.

5. The percentage of samples containing tubercle bacilli shows no decrease when the work of the last few years is compared with the first work done twenty years ago.

6. Tubercle bacilli remain virulent in butter as ordinarily salted, for more than five months.

7. Two of six samples of butter tested at this laboratory contained tubercle bacilli virulent to guinea pigs. Pages 328-333

8. There is recorded in the literature the testing of 7845 samples of market milk for tubercle bacilli of which 537, or 6.8 percent, contained this organism. In the United States three authors found tubercle bacilli present in 78, or 17.5 percent, in a total of 447 samples of milk tested, while in Continental Europe four authors found this organism present in 205, or 4.8 percent, of 4229 samples tested.

9. In 37 samples of market milk tested at this Station no tubercle bacilli were found.

10. Tubercle bacilli have been found in the milk from tuberculous cows with sound udders by many reliable investigators. These bacteria probably gained access to the milk from other sources (manure, urine, dust) than the interior of the udder, in most cases.

11. Tubercle bacilli are rarely excreted in the milk of tuberculous cows with sound udders, especially if the infection is localized; when the infection is generalized or when tubercle bacilli are *free in the blood,* these organisms may be excreted along with the milk.

12. Forty-seven samples of milk from ten tuberculous cows, tested at this laboratory, gave wholly negative results. None of the ten cows was found to be extensively tuberculous *post mortem.*

Pages 334-362

13. Guinea pig inoculation tests are superior to microscopic tests in detecting the presence of tubercle bacilli in cow manure. By inoculation tests, tubercle bacilli in a suspension of fresh cow manure can be detected when present in an amount as small as one hundred billionth of a gram in the dose injected.

14. Ordinarily only a small percentage of tuberculous cattle are excreting virulent tubercle bacilli in their feces at any given time.

15. At this Station ninety-seven samples of feces from sixty-two cattle have been tested for tubercle bacilli by inoculation of guinea pigs. These organisms have been found four times in samples from three of the cows. Pages 362-390

16. At present the results of different investigators in detecting tubercle bacilli in the circulating blood are conflicting. This difference may be due to the amount of sample injected into the test animal; the larger the amount of sample, the larger the percentage of positive findings.

17. The contention of Rosenberger that tubercle bacilli are frequently found in the blood by microscopic test has not been confirmed by other workers.

18. As a diagnostic procedure the examination of the blood for tubercle bacilli is of little value.

19. Twelve samples of blood from eight cattle were tested for tubercle bacilli, both by the Rosenberger method and by guinea pig inoculations. No test showed the presence of these organisms.

Pages 390-402

20. There are about 200,000 deaths annually in the United States from tuberculosis, of which it is estimated (Park and Krumwiede) that 92 percent are caused by the human type of tubercle bacilli; and 8 percent, or 16,000 cases, are caused by the bovine type.

21. In the cases caused by the bovine type of tubercle bacilli the disease is probably contracted very largely thru the food and is found almost exclusively among children. Pages 402-406

22. Though our knowledge concerning distribution of cattle tuberculosis is yet incomplete, it is known to be extensive thruout the world; ranging from 10 to 48 percent where most testing has been done.

23. The two things most favoring the distribution of this disease are the extensive trade in (tuberculous) cattle, and the difficulty of recognizing the disease by physical signs until it is far advanced. Pages 406-412

24. The recognition of tuberculosis in cattle depends almost entirely upon the tuberculin test. The positive test is accurate in about 98 percent of the cases as shown by slaughter. The negative test is not so reliable.

25. The Bang method of handling a tuberculous herd is recommended to owners of large herds. The modified Bang method by means of a "Stock Owners' Association," is recommended to owners of smaller herds.

26. The responsibility of ridding farm animals of tuberculosis must finally be placed upon the stock owner. The State may well formulate rules and regulations, provide for free tuberculin testing, and for popular and special education upon the subject of animal tuberculosis. Pages 412-431

Note.—The experimental and bibliographic work shown in this bulletin was done by Mr. Briscoe of the Department of Botany, which includes bacteriology. The manuscript in its original form was also prepared by him. Dr. MacNeal, Assistant Professor of Botany (bacteriology) and a member of the Station Staff, directed the work and made a careful revision of the entire matter for publication.

T. J. Burrill, *Head of Department.*

TUBERCULOSIS OF FARM ANIMALS

By CHAS. F. BRISCOE, Instructor in Botany, and W. J. MacNEAL, Assistant Chief in Bacteriology

INTRODUCTION

CAUSE

Tuberculosis is a contagious disease widely prevalent thruout the world. The infectious agent is a bacterium, *Bacillus tuberculosis*. This germ is conveyed from animal to animal, by direct contact, by means of food or drink, by inhalation, or it may gain entrance thru a wound. *Bacillus tuberculosis* is a microscopic plant. It has the shape of a very small, thin rod. It is a definite species. No tubercle bacillus can originate except it comes from a parent tubercle bacillus; any more than a white oak tree can come from any other than a parent white oak tree. No animal, no person can take this disease unless this specific germ gains entrance to the body, and by its multiplication in the body produces its characteristic effects. It should be emphasized at the outset that *Bacillus tuberculosis* is not found everywhere. It cannot live long outside the animal body under ordinary conditions. It is easily killed by light, and is killed, when fully exposed, by drying, tho it may live for weeks or even months in apparently dry material. It is not found except in places where animals or human beings with the disease live or have lately lived. Tuberculosis is a purely contagious disease and in domestic animals it is contracted very largely thru close association with a diseased animal. The disease is not inherited, tho in some rare instances the tubercle bacillus may gain entrance to the young before birth. A weak bodily resistance to the tubercle bacillus may be inherited.

HISTORICAL ACCOUNT

Tuberculosis was known to the Ancient Greeks as early as the fifth century before Christ.[2] Hippocrates gave (460 B. C.) a description of this disease, tho he surely did not understand the tubercle as we know it today. But little more was written concerning the disease until the 17th century of our era; then came the period of anatomical investigation. About the middle of the 19th century what we may call the period of experimental investigation began, and among the investigations, that of Villemin, published in December, 1865, proving the infectiousness of the disease, is of fundamental importance. Villemin inoculated rabbits subcutaneously behind the ear with pus of yellow and gray tubercles from consumptive

men and also from tuberculous cattle, and produced in this way tubercles in the lungs and livers of the rabbits. Since then Villemin's experiments have been repeated and confirmed by thousands of workers. In 1882, Robert Koch discovered the tubercle bacillus, stained, described and successfully cultivated it, and produced tuberculosis by inoculating the germ, free from all other living material, into animals. In 1890, Koch prepared and described the substance known as tuberculin which has now become so useful as a diagnostic agent.

Very early in the study of the tubercle bacilli, certain differences were discovered between those present in mammals, those of birds, and those infecting fish, and these three distinct types have been recognized for many years. In 1898, Theobald Smith first recognized type differences between the tubercle bacilli present in human pulmonary tuberculosis and those present in tuberculosis (pearl disease) of cattle. The former he called the human type, the latter, the bovine type.

The organisms belonging to the human, bovine, and avian types are by far the most important economically and of these the human and the bovine, especially in America, concern us most. These latter were distinguished by Smith,[12] by differences in their morphology, in growth in artificial culture, in the reaction produced in glycerin broth cultures, and in their virulence. The human type is less virulent for rabbits than the bovine type. The human type produces acid when grown for some weeks in five percent glycerin broth; the bovine type produces an alkalin reaction. The human type grows more readily and more abundantly than the bovine type on culture media in general. The human bacillus is longer, more slender, and curved; the bovine bacillus is shorter, plumper, and usually straighter.

VIRULENCE OF BOVINE AND HUMAN TYPES

The possible practical importance of this difference in type is at once apparent. Koch perceived this and followed up Smith's work with an extensive investigation of the transmissibility of the human disease to cattle, and arrived at the startling conclusion which he first announced at the Tuberculosis Congress at London, 1901. He said that cattle can not be infected with the bacillus of the human type and that man need have little fear of infection with the bovine organism from animal products. This statement of Koch aroused considerable opposition at the time, and has stimulated the production, during the last nine years, of a great deal of work upon the subject. The opinion of the great majority of scientists, at the Sixth International Congress on Tuberculosis at Washington in 1908, was strongly against

Koch's statement in regard to the danger of the bovine bacillus for man.

There are on record numerous observations of the infection of cattle with tubercle bacilli derived from man. These have been reviewed by Ravenel[10] who concludes that there is no longer any doubt that such transmission can take place. Schweinitz and Dorset[11] come to the same conclusion.

That man can be infected by the bovine tubercle bacilli does not, from the nature of the question, readily admit of direct experimental proof. But indirect evidence has been brought forward in abundance. Instances in which children and men have been infected by tubercle bacilli of the bovine type have been reported by a large number of investigators. Many of these instances are reviewed by Ravenel[10] and more recent reports of similar cases have been made by Hess,[5] Lewis,[7] Park,[8] Duval,[3] and Park and Krumwiede.[9] The case reported by Troje[14] may be given as an example in which the evidence was very clear. A young butcher in good health, and with no hereditary taint, wounded his right forearm while slaughtering a tuberculous cow. The wound at first healed under medical treatment but a month later proved to be tuberculous. Very many cases of tuberculosis in children have been reported where the infection had evidently come thru the ingestion of milk and other dairy products. The isolation of the bovine type of tubercle bacilli from tubercles in children, especially from the lymphatic glands of the neck and intestines, is not at all uncommon. Primary intestinal tuberculosis and tuberculosis of the lymphatics in man are frequently caused by tubercle bacilli of the bovine type. These kinds of tuberculosis are thot by Koch[6] to be relatively very infrequent. On the other hand, Fibiger and Jensen[4] think that of all tuberculous cases in children 16 percent are of this type. Park and Krumwiede[9] found in an examination of a total of 435 consecutive cases of tuberculosis (that is all cases were examined regardless of type) 32, or 7.4 percent, caused by tubercle bacilli of the bovine type. Of adults over 16 years of age only one case of 297 examined was caused by bacilli of the bovine type, while in children between the ages of 5 and 16 years, 9 out of 54 or 16⅔ percent, and in children under 5 years of age, 22 out of a total of 84 cases examined, or 26.2 percent, were found to be caused by the bovine type of tubercle bacillus. Theobald Smith[13] has estimated that about one percent of tuberculosis in man is of the bovine type. Charles F. Dawson says, "A summation of the views of various authors shows a preponderance of opinion against Koch's theory", in which he doubted that human and bovine tuberculosis are intertransmissible.

From the mass of apparently conflicting evidence in regard to the intertransmissibility of tuberculosis between the human and the bovine species, it is impossible at present to arrive at the whole truth. The following facts seem well established:

1. Healthy bovine animals do not naturally become infected with tubercle bacilli of the human type.

2. The human species can be infected with tubercle bacilli of the bovine type, and such infections appear to be rather common in children.

3. Bovine animals have been infected with tubercle bacilli derived from cases of human tuberculosis. It seems probable, in some cases at least, that these were instances of human infection with the bovine type of bacillus rather than instances of infection of cattle with the human type of the bacillus.

4. Evidence is still too slight for deciding whether the bovine type of tubercle bacillus may be, in nature, transformed into the human type.

SUMMARIZED STATEMENT

1. Tuberculosis is a disease due to a specific organism. It is contagious and transmitted from one animal to another.

2. It was known to the early Greeks, 460 B. C., but was little understood until the middle of this last century.

3. Several types of tubercle bacilli have been recognized; the human, bovine, avian, and a type belonging to the cold blooded animals.

4. The types are more or less intertransmissible from one species of animal to another.

PATHOLOGY

THE TUBERCLE

The most characteristic tissue change in the disease known as tuberculosis is the formation of tubercles. These are more especially found in the lungs, lymph glands, spleen and liver, but may be found in any organ and in almost all tissues of the body. They are not uncommon in the bones but rare in the muscles and nervous tissues. The tubercles vary in size from a millet seed, and less, to more than an inch in diameter. They are pearly white to a yellowish color. They are often hard, fibrous or calcareous but sometimes soft and full of pus.

ENTRANCE TO THE BODY

The tubercle bacilli may gain entrance to the body thru the lungs by particles breathed in, or they may be absorbed directly thru the lining membranes of the alimentary tract along with the

food and may be carried to the lymph glands or lungs by the blood and lymph currents, and wherever arrested, multiply and irritate the tissue at that point. By the resulting increase of tissue cells a knot or tubercle is formed. From this primary center, which may be single or multiple, the tubercle bacilli may be carried by means of the lymph or blood vessels to other parts of the body. When many bacilli are carried thus by means of the blood vessels to all parts of the body, there is produced generalized, miliary tuberculosis.

OPEN AND CLOSED TUBERCULOSIS

The organisms may be restricted in their multiplication and invasion, and a tough wall of tissue may form around the tubercle, shutting in the germs so they can not easily be carried to other parts of the body (Fig. 1). This is called closed tuberculosis. In other cases, the tubercles are open and cavities are formed as is often the case in the lungs (Fig. 2). Since infectious pus is continuously poured out, this condition is known as open tuberculosis.

ESCAPE OF TUBERCLE BACILLI FROM THE BODY

This infectious pus may be coughed up and smeared over the manger, fodder, or drinking trough, or it may be swallowed and passed out with the dung. Tubercle bacilli may reach the intestinal contents also from tubercles of the intestines or they may be passed out in the bile secretions directly into the intestine. In such cases, the dung contains living tubercle bacilli, and thus the barn lot and pasture may become dangerous to other cattle. In a similar way the urine or secretions from the genital organs may carry out the germs. The milk from a tuberculosis udder is a very dangerous source of infection.

APPEARANCE OF TUBERCULOUS ANIMALS

In the early stages of the disease, there are no outward manifestations; no fever, nor signs of pain; the animal may be fat and sleek, eat heartily, give plenty of milk and apparently breed as well as an animal free from tuberculosis. If the udder or neck glands are affected, the change in these may be detected earlier, but not often even then until the disease is well advanced. In these cases knots can be felt in the enlarged glands which are hard and painless. It is one of the characteristics of tuberculosis that squeezing upon the tuberculous nodule gives no pain, or much less pain than is usual for such knots found in other diseases. If the larynx, bronchi, or lungs are affected a cough is generally noticeable, especially after exercise such as running

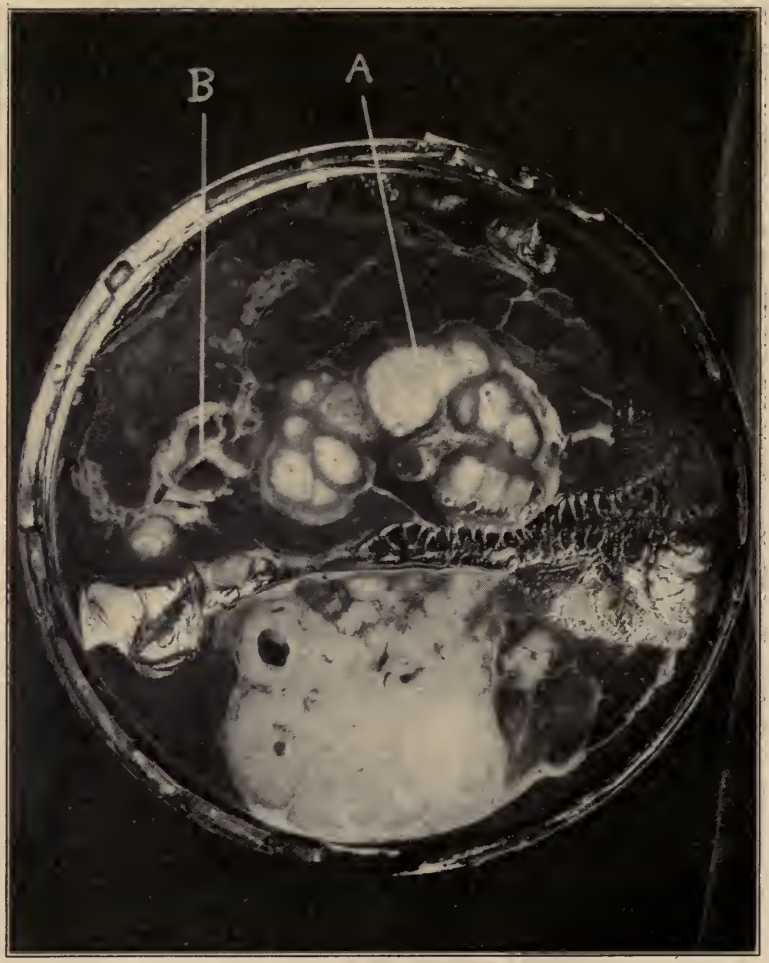

FIG. 1. SECTION THROUGH A PIECE OF LUNG FROM A COW, SHOWING CLOSED TUBERCULOSIS. ENCAPSULATED TUBERCLES AT A. BRONCHIAL TUBE AT B. NATURAL SIZE.

FIG. 2. SECTION THROUGH HUMAN LUNG SHOWING EXTENSIVE OPEN TUBERCULOSIS WITH CAVITIES.] [REDUCED. (AFTER G. CORNET, DIE TUBERKULOSE, TAFEL IV.)

around the lot for a short time. By placing the ear over the lungs an unusual sound may sometimes be heard—a crackling, bubbling, or rasping sound. The breathing is more irregular. Tuberculosis of the uterus or ovaries may cause abortion or sterility, while an involvement of the alimentary tract often causes diarrhea. As the disease advances, even tho she may continue to eat a normal amount, the cow loses flesh. The hair becomes rough and loses its luster. The bones become more prominent, the eyes sunken, the ears droop, the head and neck are carried lower, there is a decrease in milk, and the milk becomes blue and thin. In the last stage, the animal becomes stiff; in many cases she can not lie down because of the difficulty in breathing. At this stage, a high temperature is often present; progressive emaciation and loss of strength continue until death ensues. Sometimes the disease in the earlier stages may be arrested, the tubercle encapsulated, and the cow may live for some time, but complete recovery is probably rare if it really does ever occur in the bovine species.

SUMMARIZED STATEMENT

1. The most characteristic thing about tuberculosis is the formation of tubercles as a reaction on the part of the animal tissues.

2. The tubercle bacilli may enter the body thru breathing, may be taken in with food and absorbed, and thru wounds in the skin.

3. Tuberculosis is called open when the lesions are discharging tubercle bacilli, and closed when a confining membrane retains the organisms in the tubercle.

4. The tubercle bacilli leave the body thru the mouth or nose, or in the milk, feces, urine, or genital discharges.

5. Tuberculous animals in the early and moderately advanced stages appear well and active, and become emaciated only in the final stages of the disease.

GENERAL METHODS EMPLOYED IN DETERMINING THE PRESENCE OF THE BACILLUS

MICROSCOPICAL EXAMINATION

The microscopical method of examining milk, butter, feces, and urine for *Bacillus tuberculosis,* tho used to a considerable extent by early investigators, is not at present considered very reliable.

PREPARING AND STAINING THE SMEAR

To examine the material with the microscope a thin smear is made on a glass cover slip and fixed by passing it thru the flame three times and then staining with a strong red dye, hot carbol fuchsin.

This is washed in cold water and decolorized with ten percent nitric acid until only a slight pink color remains. The preparation is then dipped for ten seconds into eighty percent alcohol, and again washed in water. It is now counterstained with methylene blue; dried and mounted in balsam. The tubercle bacillus has in its outer coating a waxy layer. This layer of wax prevents ordinary applications of stain from entering it rapidly, but when heat is applied the strong carbol fuchsin penetrates the organism readily. In the cold, ten percent, or even thirty percent nitric acid will not decolorize the tubercle bacillus, but will decolorize all other organisms and tissues on the slide. After the acid is all washed out with water, the other material on the slide can be stained with the methylene blue solution. So in this preparation the tubercle bacillus appears as a thin, somewhat curved, rod, stained deep red, which contrasts sharply with the other material on the slide which takes the blue stain.

RELIABILITY

Other acid-fast organisms, however, take the same stain and can not be distinguished from the true tubercle bacillus by their appearance. Some of these may be eliminated by decolorizing with alcohol. Bang[1] recommends microscopical examinations only for rapid work, as in the case of udder tuberculosis where hundreds of organisms may be found on a slide. It is not at all reliable where only a few are present in the material to be examined. By this method of examination, the true tubercle bacillus can not be accurately distinguished from other acid-fast organisms, such as the butter bacillus of Rabinowitsch or the Timothy grass bacillus of Moeller, and besides, one often fails to detect it when present in small numbers. A more reliable test for the presence of the bacillus is animal inoculation. From two to ten cubic centimeters of material may be examined by inoculation into a guinea pig; but if this material were spread out thin enough for microscopical examination it would make a surface so large as to take some days to examine it with a microscope.

ANIMAL INOCULATION

STOCK ANIMALS

For our inoculations, guinea pigs alone have been used. They were bought with care, using only young, unused animals, weighing about 200 grams when purchased. When inoculated they weighed from 250 to 300 grams. In stock they were kept in pens, about 10 to 25 in a pen. Watering and feeding pans were scalded or steamed daily. The animals were fed clover hay, carrots, sugar-beets, and corn meal.

CARE OF INOCULATED ANIMALS

Those inoculated from each sample were, after inoculation, kept together in a closed galvanized wire cage. They were bedded with sawdust or shavings which was changed frequently to keep the cage in good sanitary condition. Watering dishes were cleaned and steamed daily. The animals were fed twice and watered once a day. They were weighed weekly and notes on their condition made as to loss of weight, size of the lymphatic glands in the groin, axilla, any lesion at the point of inoculation, or any condition varying from the normal.

TESTING WITH TUBERCULIN

At the end of six (or more) weeks each guinea pig was injected subcutaneously with two cubic centimeters of tuberculin, following the method of Anderson, who claims that this will detect not only the presence of tuberculosis but will distinguish between true and pseudo-tuberculosis. In our work, we have found no pseudo-tuberculous organisms. But the tuberculin has never failed either to kill or make very sick any guinea pig that had tuberculosis. It had none or but slight effect upon those free from the disease. We have so far injected 142 guinea pigs with the tuberculin; thirteen of these had tuberculosis and all but one of these thirteen died in less than twenty-four hours after the injection. This one was made very sick and was killed by chloroform twenty-four hours after the injection and found to contain tuberculous lesions. The 129 having no tuberculosis as shown by autopsy were not visibly affected by the tuberculin. In the pigs free from tuberculosis autopsy often revealed a slight reddening at the point of injection of tuberculin, and in some cases a considerable swelling, but these guinea pigs apparently did not notice any inconvenience from the two cubic centimeters of tuberculin injected. They ate and behaved otherwise as usual.

POST MORTEM EXAMINATION

Twenty-four hours after injecting with tuberculin *post mortem* examination was performed on all the guinea pigs. Those not already dead from the tuberculin were chloroformed and all were carefully examined for tuberculosis. If any suspicious tissues were discovered, stained preparations were made and examined microscopically, tubes of egg medium were inoculated, tissues were fixed for sectioning, and two other guinea pigs were inoculated with an emulsion of the suspected tissue. If we obtained from all these tests results characteristic of the disease, we could be fairly sure that the organism in question was *Bacillus tuberculosis*. Such procedure is, however, necessary in order to distinguish from pseudo-tuberculous and other acid-fast organisms.

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EXAMINATION OF BUTTER FOR TUBERCLE BACILLI

LITERATURE

EXPLANATION OF TABLES

A detailed review of the literature on the examination of butter for tubercle bacilli would be too extensive for the purposes of this bulletin, so a tabulation of it is given instead. See Table 1. The references are cited by numbers which refer to the authors arranged alpha-

TABLE 1.—BACILLUS TUBERCULOSIS IN MARKET BUTTER

No.	Author	Date	Place	Samples examined	Samples positive	Percent positive	Remark
1	Brusaferro[7]	1890	Turin	9	1	11.1	
2	Roth[30]	1894	Zürich	20	2	10.0	Microscopic method
3	Obermüller[24]	1895	Berlin	13	8	61.0	
4	Schuchardt[33]	1896	Marburg	42	0	00.0	
5	Obermüller[23]	1897	Berlin	14	14	100.0	16 tested, 2 lost
6	Gröning[11]	1897	Hamburg	17	8	47.0	
7	Himesch[15]	1897	Wien	?	0	0.0	Reported by Markl
8	Rabinowitsch[28]	1897	Berlin	30	0	0.0	
9	Rabinowitsch[28]	1897	Philadelphia	50	0	0.0	
10	Petri[26]	1897	Berlin	102	33	32.3	
11	Horman and Morganroth[16]	1897	Berlin	10	3	30.0	
12	Rabinowitsch[27]	1899	Berlin	15	2	13.3	First series
13	Rabinowitsch[27]	1899	Berlin	?	?	87.2	Second series
14	Rabinowitsch[27]	1899	Berlin	15	15	100.0	Third series
15	Rabinowitsch[27]	1899	Berlin	19	0	0.0	Fourth series
16	Obermüller[22]	1899	Berlin	10	4	40.0	
17	Korn[18]	1899	Freiburg	17	4	23.5	
18	Ascher[4]	1899	Königsberg	27	2	7.4	
19	Jäger[17]	1899	Königsberg	3	1	33.3	
20	Coggi[8]	1899	Milan	100	12	12.0	
21	Weissenfeld[37]	1899	Bonn	32	3	9.4	
22	Grassberger[10]	1899	Wien	10	0	0.0	
23	Herbert[13]	1899	Tübingen	43	0	0.0	
24	Herbert[18]	1899	Württemberg	58	0	0.0	Pseudo-tuberculosis 5 percent
25	Herbert[13]	1899	Berlin	20	0	0.0	Pseudo-tuberculosis 8 percent
26	Herbert[13]	1899	München	5	0	0.0	Pseudo-tuberculosis 4 percent
27	Abenhausen[1]	1900	Marburg	39	0	0.0	
28	Hellström[12]	1900	Helsingfors	8	1	12.5	12 samples, 4 lost
29	Bonhoff[6]	1900	Marburg	28	0	0.0	39 samples, 11 lost
30	Pawlowsky[25]	1900	Kiew	23	1	4.3	
31	Tobler[36]	1901	Zürich	12	2	16.7	
32	Lorenz[19]	1901	Dorpat	30	0	0.0	
33	Markl[20]	1901	Wien	43	0	0.0	
34	Herr und Beninde[14]	1901	Breslau	52	6	11.1	Two are doubtful
35	Aujeszky[3]	1902	Budapest	17	3	17.6	
36	Thu[35]	1902	Christiana	16	0	0.0	
37	Teichert[34]	1904	Rosen	40	12	30.0	
38	Reitz[29]	1906	Stuttgart	94	8	8.5	Butter from 88 dairies
39	Eber[9]	1908	Leipsic	150	18	12.0	
	Totals			1233	163	13.2	

betically in the bibliography at the close of this section. In the table, the number of samples examined, the number in which tubercle bacilli were found, and the percent of positive findings are indicated. The examinations were always performed by the method of animal inoculation, except where otherwise noted in the column headed, "Remark". By the authors cited in this table (Table 1), 1233 samples of market butter were examined, of which 163 were found to contain tubercle bacilli, or 13.2 percent of the total.

GENERAL CONSIDERATIONS

There are great differences between the findings of different investigators, which may be due to the different sources of their material. In some cases different methods of work may have had a part also in determining the results obtained. There is no evident progressive change in the percentage of infected samples during the period covered by these examinations.

In this connection it may be of interest to note that tubercle bacilli have also been found in oleomargarine. In Table 2 are given the results obtained by seven investigators in the examination of 209 samples, of which 9, or 4.3 percent, were found to contain tubercle bacilli.

TABLE 2.—BACILLUS TUBERCULOSIS IN OLEOMARGARINE

No.	Author	Date	Place	Samples examined	Samples positive	Percent positive	Remark
1	Morganroth[21]	1899	Berlin	10	8	80.0	
2	Annette[2]	1900	Berlin	15	0	0.0	
3	Annette[2]	1900	Liverpool	13	1	8.0	Nearly 8 percent
4	Bonhoff[6]	1901	Marburg	3	0	0.0	7 samples, 4 lost
5	Markl[20]	1901	Wien	3	0	0.0	
6	Thu[35]	1902	Christiana	15	0	0.0	From 4 factories
7	Eber[9]	1908	Leipsic	150	0	0.0	
	Totals			209	9	4.3	

There has been very little work in the United States on the examination of market butter for tubercle bacilli. Schroeder and Cotton[32] have made a study of the length of time that *Bacillus tuberculosis* will live in butter as ordinarily salted for the market, i. e. one ounce of salt to the pound of butter. They found by means of guinea pig inoculations that the tubercle bacilli in butter retained their virulence for 133 days at least. Schroeder[31] in a

report upon further studies on the viability of tubercle bacillus in salted butter shows that it remains virulent to guinea pigs for 160 days or longer. He also reports the feeding of tuberculous butter to four hogs. The butter was three months old. Each hog received one ounce daily mixed with its feed. Three of the four hogs became tuberculous.

METHOD OF PROCEDURE

The method usually employed in the examination of butter for tubercle bacilli is as follows. Butter is bought in the market, taken to the laboratory and placed in a sterile dish. The piece is separated into two parts with a sterile knife, and from the central portion of the freshly cut surface a sufficient amount to use in the test is removed with a sterile spatula and is placed in a sterile, glass-stoppered bottle. This bottle is placed in a 38° incubator until the butter is melted, when it is injected subcutaneously into guinea pigs, a dose of from 1 to 5 cc. being given. In order to detect a small number of tubercle bacilli, the melted butter should be centrifuged and the sediment injected. In this way the bacterial content of a larger amount of material can be placed under the skin of the test animal. The sediment may also be examined microscopically.

EXPERIMENTAL WORK ON BUTTER

SAMPLES EXAMINED

Only eight samples of butter have been examined in our laboratory. The samples were all obtained from the University Experiment Station Creamery. The milk from which the butter was made came from the tuberculous herd of the University Experiment Station and from farmers' herds in the surrounding neighborhood. The samples of butter were furnished us directly from the moulding board and were put into 3-ounce sterile, glass-stoppered, wide-mouthed bottles. The butter was melted and three cubic centimeters inoculated intraperitoneally into each of three guinea pigs. The results are shown in Table 3.

RESULTS

Of the eight samples two were lost by the guinea pigs dying one day after inoculation. Of the six samples completing the test, two, or 33⅓ percent, contained tubercle bacilli. It should be said that a number of samples of milk from the University herd have since been examined and none of these has proved to be tuberculous; it therefore seems probable that the tubercle bacilli came from some one of the farmers' herds.

TABLE 3.—TUBERCLE BACILLI IN BUTTER FROM THE UNIVERSITY DAIRY

Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic examination	Cultures	Re-inoculation
1	3	7-3-'09	Killed	67	Normal			
	4	7-3-'09	Killed	67	Normal			
	5	7-3-'09	Killed	67	Generalized tuberculosis in the lymphatics, lungs, liver, spleen, etc.	+	+	++
2	6	7-6-'09	Killed	65	Tuberculous only in the right superficial inguinal lymph gland	+	+	++
	7	7-6-'09	Died	10	Acute infection	*	*	
	8	7-6-'09	Killed	65	Generalized tuberculosis in the liver, spleen, mesentery, diaphragm, lymph glands, etc.	+	+	++
3	24	7-28-'09	Died	1	Acute infection			
	25	7-28-'09	Died	1	Acute infection			
	26	7-28-'09	Died	1	Acute infection			
4	27	7-31-'09	Died	1	Acute infection			
	28	7-31-'09	Died	1	Acute infection			
	29	7-31-'09	Died	1	Acute infection			
5	33	8- 8-'09	Killed	59	Normal			
	34	8- 8-'09	Killed	59	Normal			
	35	8- 8-'09	Killed	59	Normal			
6	63	8-14-'09	Killed	55	Normal			
	64	8-14-'09	Killed	55	Normal			
	65	8-14-'09	Killed	55	Normal			
7	90	8-21-'09	Killed	65	Normal			
	91	8-21-'09	Died	1	Acute infection			
	92	8-21-'09	Killed	65	Normal			
8	108	8-28-'09	Died	9	Acute infection			
	109	8-28-'09	Killed	62	Large tumor at the point of inoculation. No tubercles. Organs normal.	†	†	Normal
	110	8-28-'09	Killed	62	Normal			

*Micrococci.

†Several kinds of bacteria present.

Summary: Eight samples, two lost; of the other six, two contained tubercle bacilli, 33⅓ percent

CONCLUSIONS

1. *Bacillus tuberculosis* is widely distributed in market butter.

2. The examinations in the last few years show no decrease in the percentage of samples containing tubercle bacilli as compared with the first work twenty years ago.

3. *Bacillus tuberculosis* remains alive in butter as ordinarily salted for a much longer time than butter is usually kept in storage.

4. Butter containing tubercle bacilli, when fed to hogs, may produce generalized tuberculosis.

5. Of the six samples of butter tested by us, two, or 33⅓ percent, contained tubercle bacilli virulent to guinea pigs.

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EXAMINATION OF MARKET MILK FOR TUBERCLE BACILLI

LITERATURE

The literature relating to the tubercle bacillus in milk is very extensive and we must content ourselves with giving it, in the main, in tabular form, as in the case of market butter. The citations are made in these tables in the same way as they were in Table 1.

In these tables we have attempted to separate the literature concerning the presence of tubercle bacilli in market milk from that reporting the work done on milk of tuberculin-reacting cows with sound udders, a distinction not always made by the authors. For this reason, it may be that some items are misplaced in the tables, but in the main they are correct and it is worth while to make the distinction. There are two distinct questions that have called forth this great amount of work. On the one hand, the investigators have sought to determine the percentage of the samples of milk found on the market containing tubercle bacilli, and on the other hand, other investigators have tried to determine whether a cow which evidently reacts to tuberculin, but which has sound udders, will give milk containing tubercle bacilli. Table 4 gives the literature on the bacilli in market milk.

TABLE 4.—BACILLUS TUBERCULOSIS IN MARKET MILK

No.	Author	Date	Place	Samples examined	Samples positive	Percent positive	Remark
1	Martin[27]	1884	Paris	9	3	33.3	12 samples, 3 lost
2	Ernst[13]	1890	Boston	33	(1)	3.3	Microscopic
3	Ernst[13]	1890	Boston	(13)	3	23.1	Animal inoculation
4	Gebhardt[16]	1890	Munich	10	0	0.0	
5	Sacharbekow[37]	1893	St. Petersburg	80	4	5.0	
6	Friis[15]	1893	Copenhagen	28	4	14.3	City milk
7	Friis[15]	1894	Copenhagen	33	0	0.0	Country
8	Schroeder[38]	1894	Washington	19	1	5.3	
9	Chester[10]	1895	Newark	30	0	0.0	
10	Obermüller[30]	1895	Berlin	13	8	61.5	
11	Buege[8]	1896	Halle	6	2	33.3	9 samples, 3 lost
12	Hope[18]	1897	Liverpool	228	12	5.2	City milk
13	Hope[18]	1897	Liverpool	67	9	13.4	Country
14	Rabinowitsch and Kempner[36]	1897	Berlin	25	7	28 0	
15	Boyce[5&6]	1897	Liverpool	144	4	2.8	City milk
16	Boyce[5&6]	1897	Liverpool	24	7	29 1	Country milk
17	Massone[28]	1897	Genoa	(?)	(?)	9.0	
18	Kudinow[22]	1898	Dorpat	(?)	(?)	2.5	
19	Petri[33]	1898	Berlin	57	9	17.5	Corrected figures

TABLE 4.—*Continued*

No.	Author	Date	Place	Samples examined	Samples positive	Percent positive	Remark
20	Ott[31]	1898	Schw. Gmünd	43	5	11.6	Microscopic
21	Ott[31]	1898	Schw. Gmünd	(27)	(3)	11.1	Animal inoculation
22	Delépine[11]	1898	Liverpool	54	3	5.5	City milk
23	Delépine[11]	1898	Liverpool	125	22	17.6	Country milk
24	Boyce[5]	1898	Liverpool	84	7	8.3	City milk
25	Boyce[5]	1898	Liverpool	28	5	17.9	Country
26	Boyce[5]	1899	Liverpool	75	5	6.6	City milk
27	Boyce[5]	1899	Liverpool	63	11	17.4	Country
28	Ascher[2]	1899	Königsberg	17	0	0.0	Reacting cows
29	Jaeger[19]	1899	Königsberg	100	7	7.0	Microscopic
30	Macfadyen[23]	1899	England	77	17	22.1	100 samples, 23 lost
31	Kanthack and Sladen[20]	1899	Cambridge	16	9	56.3	
32	Woodhead and Wood[42]	1899	Liverpool	50	5	10.0	
33	Marconi[25]	1900	Naples	12	6	50.0	14 samples, 2 lost
34	Marconi[25]	1900	Naples	14	7	50.0	Second series
35	Marshall[26]	1900	Lansing, Mich.	50	1	2.0	
36	Rabinowitsch[35]	1900	Berlin	3	0	0.0	Tested cows
37	Rabinowitsch[35]	1900	Berlin	5	3	60.0	Cows not tested
38	Klein[21]	1900	London	100	7	7.0	8 pseudo-tuberculosis
39	Pawlowsky[32]	1900	Kiew	51	1	2.0	
40	Bujwid[9]	1900	Krakow	60	2	3.3	
41	Beck[4]	1900	Berlin	56	17	30.3	15 pseudo-tuberculosis
42	Magill[24]	1901	Paris	(?)	(?)	12.0	
43	Magill[24]	1901	New York	(?)	(?)	11.0	
44	Tonzig[41]	1901	Padua	38	0	0.0	
45	Thu[40]	1902	Christiana	44	0	0.0	1 pseudo-tuberculosis
46	Müller[29]	1904	Germany	1596	97	6.2	59 tuberculous udders
47	Beatty[3]	1904	Germany	272	27	10.0	14 tuberculous udders
48	Smit[39]	1905	Rotterdam	567	14	2.7	
49	Smit[39]	1906	Rotterdam	1584	45	2.8	
50	Brittlebank[7]	1906	Manchester	764	some	?	From 565 farms
51	Proskauer, Seligmann and Croner[34]	1907	Denmark	13	5	38.5	1 pseudo-tuberculosis
52	Proskauer, Seligmann and Croner[34]	1907	Berlin	9	5	55.5	
53	Proskauer, Seligmann and Croner[34]	1907	Berlin	5	0	0.0	Inspected dairies
54	Anderson[1]	1908	Washington	223	15	6.7	272 samples, 49 lost
55	Eber[12]	1908	Leipsic	210	22	10.5	Milk
56	Eber[12]	1908	Leipsic	50	3	6.0	Cream
57	Eber[12]	1908	Leipsic	50	2	4.0	Cottage cheese
58	Field[14]	1909	Louisville	119	46	39 5	
59	Hess[17]	1909	New York	105	17	16.2	
	Microscopic tests			176	13	7.4	
	Animal inoculation tests			7397	502	6.8-	
	Totals			7573	515	6.8+	

The determination of tubercle bacilli in milk is not a very difficult problem. In this no attempt is made to find the source from which the bacilli entered the milk; whether from tuberculous cows or from tuberculous persons handling the milk; whether from the infected udder or from infected manure or dirt falling into the milk at milking time or otherwise. Most tests have been made by animal inoculation, and this to a large extent insures that the organisms found here have been true tubercle bacilli.

As may be seen in Table 4, work was done upon this subject as early as 1884 by Martin. The first extensive work carried out in the United States was by Ernst in 1890. More extensive investigations have been made by the boards of health of Liverpool and London, the work being reported by Hope, Delépine, Boyce, and others. More especial mention may be made of the results of Mueller and Beatty, who for several years carried out tests for the East Prussian Herdbook Society. They examined bacteriologically the mixed milk of dairy herds as a whole. Their purpose was to discover and remove from the herd, cows with tuberculous udders. They thot that in this way they would be able to prevent most of the infection by tubercle bacilli in dairy products. In this work Mueller has examined 1596 samples of milk and found 97, or 6.2 percent, containing tubercle bacilli. Beatty examined 272 samples and found 27, or 10 percent, containing these organisms. Further details of their paper will be found in the next section of this bulletin.

Anderson has examined milk from 102 dairies supplying the City of Washington. The herds supplying milk to this city contained considerable numbers of tuberculous cows as recent tuberculin tests (1907) by Mohler had shown 214 reacting animals out of 1147 tested, or 18.6 percent. Other tests by the District Health Department upon 1095 cows in 51 herds in Virginia, Maryland, and the District of Columbia, also supplying milk to the City of Washington, detected 160 reacting animals, or 15.1 percent. Anderson considers these percentages considerably below the actual amount of tuberculosis in the dairy cows supplying that city, because, he says, only the owners of herds supposed to be free from tuberculosis permitted the tests to be made. The author collected his samples in one-pint bottles with untampered stoppers. Fifty cubic centimeters of milk were added to one hundred cubic centimeters of water (to decrease the specific gravity) and the mixture was centrifuged for one hour at 2000 revolutions per minute. One guinea pig was inoculated subcutaneously with 5 cc. of the sediment. The eight guinea pigs (usually only eight samples were tested per day) were placed together in the same cage, those that remained healthy being controls on their environment. Many animals died of acute infections with other bacteria in the milk but

no attempt was made to identify any but the tubercle bacilli. In order to distinguish a tuberculous from a non-tuberculous guinea pig, 2 cc. of crude tuberculin was injected subcutaneously into each animal. This killed or made very sick any guinea pig that had tuberculosis. About 250 guinea pigs received the tuberculin in this way and no animal died that did not have tuberculosis. Two or three animals that had slight lesions did not die, but became very sick. All animals died whose lesions had caseated. The author thinks the procedure a distinct help in eliminating infections with other acid-fast organisms. Samples from 102 dairies were tested. The milk from 11, or 10.7 percent, of these 102 dairies contained tubercle bacilli. Of the 272 samples of milk tested, 49, or 18 percent, were lost by the test animal dying too early. Of the 223 samples remaining, 15, or 6.72 percent, were tuberculous.

Hess studied the milk supply of New York City with three questions in mind:

1. What percentage of market milk in a large city like New York contains tubercle bacilli?

2. What percentage of the tubercle bacilli in milk is of bovine and what is of human origin?

3. What effect has this tuberculous milk upon children?

The samples were taken from large 40-quart cans, and not from bottle-milk. This was done because more than half the milk sold in New York City is not bottled but is the so-called "loose milk." The milk was collected in one-ounce, tight fitting, tin-capped bottles. It was taken indiscriminately from grocery stores, dairies, and large plants in various quarters of the city. Usually the milk was collected by the investigator himself. Each sample was tested by microscopic examination of stained preparations and by the inoculation of two guinea pigs. The milk was centrifuged and the cream dipped off with a sterile copper spoon and placed in a sterile Petri dish. The "skim milk" was then poured off. Microscopic preparations were made from the sediment and the cream. These were then mixed and injected into the animals. Of 112 samples tested, 5 were lost—the guinea pigs dying before sufficient time had elapsed to detect tubercle bacilli—and 2 were duplicates. The remaining 105 (the author says 107) gave 17 positive tests, or 16.2 percent (author gives 16 percent). There were also examined 8 samples of commercial pasteurized milk, and of these one was found to contain tubercle bacilli. The dealers stated that the milk had been pasteurized at 160° F. for forty seconds.

In order to answer the second question, whether the tubercle bacilli found in the milk are of the human or the bovine type, eight cultures were isolated and worked with. In two other cases this

was attempted, but the cultures failed. Of these, seven conformed to the bovine type and one culture conformed to the human type.

The author had collected samples especially from such dealers as had children and gave their children raw milk from the cans in the store. Of the 17 dealers selling milk which contained tubercle bacilli, 10 belonged to this category. These ten milk dealers had eighteen children. Sixteen of these children were tested with tuberculin; four reacted. One of these cases was of especial interest. This child consumed daily a pint of the poorest milk examined. She was two years old, poorly nourished, and five months before had developed a cervical adenitis, which was incised at a dispensary. No other member of the family appeared to be tuberculous. Recently the author was able to collect 44 human cases of undoubted bovine tuberculous infection of the mesenteric glands, 41 of which occurred in children. He thinks that the relation of bovine tuberculosis to man may be fairly stated as follows: 1. The bovine type of tubercle bacillus, altho less virulent for man than the human variety, is capable of infecting human beings. 2. Children are more susceptible than adults to bovine infection.

EXPERIMENTAL

COLLECTION OF SAMPLES

Champaign and Urbana are practically one city and most of the milkmen deliver milk in both towns. The samples were bought on the streets as the milkmen were delivering milk to their customers. They were all collected in pint or quart bottles with untampered stoppers. After bringing the bottle to the laboratory, it was well shaken.

TESTING OF SAMPLES

Eighty cubic centimeters were placed into four sterile centrifuge tubes and these were centrifuged for thirty minutes at 1800 revolutions per minute. Then the milk was drawn from the middle of the tube with a Pasteur bulb pipette and the cream and sediment of the four tubes put into one tube; this was again centrifuged thirty minutes at 2000 to 3000 revolutions per minute. The middle portion was drawn off from this tube and the remaining cream and sediment of the 80 cc. of milk was injected subcutaneously into three guinea pigs in graded doses, usually four, three, and one and one-half cubic centimeters respectively. In a number of cases one or two of the guinea pigs died too early to test the presence of tubercle bacilli; but in only two cases did all the guinea pigs from any one sample die too early. Thirty-seven of the thirty-nine samples completed the test. Tubercle bacilli were not found in any sample. The results are recorded in Table 5.

TABLE 5.—TUBERCLE BACILLI IN MARKET MILK OF CHAMPAIGN AND URBANA

Dairy number	Sample number	Guinea pig number	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Tuberculosis
1	1	329	3-16-'09	Died	2	Acute infection	....
		330	"	Killed	63	Normal	0
		331	"	Killed	63	Normal	0
	2	387	4-26-'09	Killed	37	Normal	0
		388	"	Died	1	Acute infection	....
		389	"	Died	2	Acute infection	....
	3	413	6-10-'09	Died	26	No tubercles	0
		414	"	Killed	43	Normal	0
		415	"	Died	26	No tubercles	0
	4	443	6-18-'09	Died	18	No tubercles	0
		444	"	Killed	42	Normal	0
		445	"	Killed	42	Normal	0
2	5	332	3-18-'09	Died	24	No tubercles	0
		333	"	Killed	61	Normal	0
		334	"	Died	16	Acute infection	0
	6	398	6-4-'09	Died	3	Acute infection	....
		399	"	Died	2	Acute infection	...
		400	"	Died	1	Acute infection	....
	7	410	6-10-'09	Killed	43	Normal	0
		411	"	Died	27	No tubercles	0
		412	"	Died	27	No tubercles	0
3	8	341	3-29-'09	Killed	50	Normal	0
		342	"	Killed	50	Normal	0
		343	"	Died	11	Acute infection	....
	9	395	6-4-'09	Died	38	No tubercles	0
		396	"	Killed	49	Normal	0
		397	"	Killed	49	Normal	0
	10	428	6-17-'09	Died	26	No tubercles	0
		429	"	Killed	43	Normal	0
		430	"	Killed	43	Normal	0
4	11	350	3-30-'09	Died	46	No tubercles	0
		351	"	Died	11	No tubercles	....
		352	"	Killed	49	Normal	0
	12	401	6-4-'09	Killed	49	Normal	0
		402	"	Died	32	No tubercles	0
		403	"	Killed	49	Normal	0
	13	533	8-31-'09	Killed	43	Normal	0
		534	"	Killed	43	Normal	0
		535	"	Died	4	Acute Infection	....
5	14	344	3-29-'09	Died	14	No tubercles	0
		345	"	Died	17	No tubercles	0
		346	"	Killed	50	Normal	0
	15	437	6-17-'09	Killed	43	Normal	0
		438		Killed	43	Normal	0
		439		Killed	43	Normal	0
6	16	353	3-30-'09	Killed	49	Normal	0
		354		Killed	49	Normal	0
		355		Died	2	Acute infection	...
	17	404	6-9-'09	Died	5	Acute infection	...
		405	"	Killed	44	Normal	0
		406	"	Killed	46	Normal	0
7	18	416	6-11-'09	Killed	42	Normal	0
		417	"	Died	23	No tubercles	0
		418	"	Killed	42	Normal	0
	19	452	6-21-'09	Died	21	No tubercles	0
		453	"	Died	21	No tubercles	0
		454	"	Died	21	No tubercles	0

TABLE 5.—*Continued*

Dairy number	Sample number	Guinea pig number	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Tuberculosis
8	20	419	6-11-'09	Died	24	No tubercles	0
		420	"	Killed	42	Normal	0
		421	"	Killed	42	Normal	0
	21	458	6-22-'09	Killed	44	Normal	0
		459	"	Died	2	Acute infection	.
		460	"	Killed	44	Normal	0
9	22	422	6-11-'09	Killed	42	Normal	0
		423	"	Killed	42	Normal	0
		424	"	Died	27	No tubercles	0
	23	455	6-21-'09	Killed	45	Normal	0
		456	"	Killed	45	Normal	0
		457	"	Died	15	Acute infection	0
10	24	425	6-11-'09	Died	6	Acute infection	....
		426	"	Killed	49	Normal	0
		427	"	Killed	49	Normal	0
	25	536	8-31-'09	Died	3	Acute infection	....
		537	"	Died	3	Acute infection	...
		538	"	Killed	43	Normal	0
11	26	434	6-17-'09	Died	23	No tubercles	0
		435	"	Died	22	No tubercles	0
		436	"	Killed	43	Normal	0
	27	467	6-23-'09	Died	1	Acute infection	....
		468	"	Died	13	Acute infection	....
		469	"	Killed	43	Normal	0
12	28	446	6-18-'09	Died	18	No tubercles	0
		447	"	Killed	42	Normal	0
		448	"	Killed	42	Normal	0
	29	464	6-22-'09	Killed	44	Normal	0
		465	"	Killed	44	Normal	0
		466	"	Killed	44	Normal	0
13	30	407	6-9-'09	Died	26	No tubercles	0
		408	"	Killed	44	Normal	0
		409	"	Died	26	No tubercles	0
	31	470	6-23-'09	Died	13	Acute infection	....
		471	"	Died	13	Acute infection	....
		472	"	Died	13	Acute infection	....
14	32	347	3-29-'09	Killed	50	Normal	0
		348	"	Killed	50	Normal	0
		349	"	Died	5	Acute infection	....
15	33	431	6-17-'09	Killed	43	Normal	0
		432	"	Died	23	No tubercles	0
		433	"	Killed	43	Normal	0
16	34	440	6-18-'09	Killed	42	Normal	0
		441	"	Killed	42	Normal	0
		442	"	Killed	42	Normal	0
17	35	449	6-21-'09	Died	15	Acute infection	0
		450	"	Died	21	Acute infection	0
		451	"	Killed	39	Normal	0
18	36	461	6-22-'09	Died	2	Acute infection	....
		462	"	Died	2	Acute infection	....
		463	"	Killed	44	Normal	0
19	37	358	4-2-'09	Killed	48	Normal	0
		359	"	Died	8	Acute infection	....
		360	"	Killed	48	Normal	0
20	38	361	4-2-'09	Died	8	Acute infection	....
		362	"	Killed	46	Normal	0
		363	"	Died	9	Acute infection	....
21	39	384	4-26-'09	Killed	37	Normal	0
		385	"	Died	1	Acute infection	....
		386	"	Died	2	Acute infection	

CONCLUSIONS

1. According to the literature reviewed, tubercle bacilli are very common in market milk, being found, in the 7845 samples tabulated in Table 4, 537 times, or 6.8 percent.

2. A composite of the three largest testings done in the United States in 1908 and 1909, those of Anderson (Washington), Fields (Louisville), and Hess (New York City), gives a total number of 447 samples, of which the number containing tubercle bacilli is 78 or 17.5 percent. The four largest series of tests in Continental Europe, those of Mueller, Beatty, Smit and Eber, comprise a total of 4229 samples, and of these only 205 or 4.8 percent contained tubercle bacilli.

3. The testing of thirty-seven samples of market milk from Champaign and Urbana, from twenty-one dairies, revealed no tubercle bacilli.

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EXAMINATION OF MILK FROM TUBERCULOUS COWS WITH SOUND UDDERS

LITERATURE

GENERAL CONSIDERATIONS

Few of the earlier investigations concerning tubercle bacilli in milk of tuberculous cows with sound udders are free from objection. The ease with which milk is contaminated from outside sources was not then appreciated, as it is today. While in the case of market milk the problem is to determine only the presence or absence of tubercle bacilli in the milk regardless of the source of infection, here it is desired to know whether or not the sound udders of tuberculous cows secrete tubercle bacilli along with the milk. So it is necessary to prevent any contamination from outside sources, such as the falling into the milk of manure infected with tubercle bacilli, infected urine, dust of the cow stable, or any contaminated

material whatsoever. The many possible sources of contamination of the milk with tubercle bacilli make the problem a very difficult one. The difficulty of reaching a conclusion is further indicated by the fact that the investigators reporting negative results and those reporting positive results are pretty equally divided.

INVESTIGATORS REPORTING NEGATIVE RESULTS

It will be seen by examining the literature, Tables 6 and 7, that most of the results are positive until we come to Ostertag's work in 1899. Ostertag took extra precautions to prevent contaminations, carefully grooming the cow, washing the udder with antiseptics, and drying with sterile wadding; also taking care to have the milker clean and disinfect his hands. After rejecting the first milk, the sample to be examined was drawn into a sterile flask. He found on testing 50 cows which had reacted to tuberculin that 49 were giving milk entirely free from tubercle bacilli, according to his test. In the case of one cow the evidence was not so conclusive. Of four guinea pigs, inoculated with her milk, one became tuberculous. Ostertag concluded, however, that this infection was due to accidental contamination.

Since Ostertag's work, McWeeney[53], Stenström[63], Ascher[2], Müller[36], and Smit[60] have done some very careful work with entirely negative results. The work of the last two authors was very extensive and will be briefly discussed. Müller examined the mixed milk from herds in order to detect the presence of cows with tuberculous udders, as has been mentioned under market milk. Of 1596 samples tested, Müller found 97, or 6.2 percent containing tubercle bacilli. When he found a tuberculous sample from a herd, an inspector was sent to examine the udders of each cow in this herd. In 59 of the 97 cases, tuberculous udders were found; in 56 of these 59 cases, only one tuberculous udder was found in each herd; in the other three cases, two tuberculous udders in each herd were found. Of the other 38 (author by mistakes says 32) samples, 19 were found to be from herds containing cows suffering from pulmonary tuberculosis; twelve from herds containing one or several cases of uterine tuberculosis. In another positive sample the bacilli were derived from an animal affected with pulmonary tuberculosis and several cases of intestinal tuberculosis in the herd. For the remaining five (two not accounted for) positive findings, there was no explanation found and other examinations of the milk of these five herds gave always negative results. But what are we to say of the 1499 herds where the milk showed no tubercle bacilli? Müller assumed that 30 to 40 percent of the nearly 20,000 dairy cows in these herds were tuberculous, i. e., 6000 to 8000 cows. This is in accordance with the statistics given for that region. Now according to the views of some authors, the 6000 to 8000 tuberculous

TABLE 6.—BACILLUS TUBERCULOSIS IN THE MILK OF TUBERCULOUS COWS WITHOUT RECOGNIZABLE DISEASE OF THE UDDER

No.	Author	Date	Place	Cows tested	Cows positive	Percent positive	Remark
1	Zurn[66]	1872	Jena	1	1	100.0	Fed to swine
2	Klebs[27]	1873	Leipsic	1	1	100.0	Fed to test animals
3	Semmer[59]	1875	Leipsic	(?)	(?)	(?)	45.6 percent. of test animals positive
4	Bollinger[8]	1880	Germany	1	1	100.0	Fed 3 hogs, 1 positive
5	Bollinger[8]	1880	Germany	1	1	100.0	Fed 5 hogs, all positive
6	Johne[26]	1883	(Compilation)	91*	28*	30.7*	
7	May[32]	1883	Munich	5	0	0.0	
8	Imlach[25]	1884		3	(?)†	(?)†	
9	Stein[62]	1884	Berlin	14	4	28.5	1 udder tuberculous *post mortem*
10	Zschokke[65]	1884	Germany	1	1	100.0	
11	Hirschberger[24]	1889	Munich	20	11	55.5	
12	Bang[5]	1889	Denmark	21	2	9.5	Normal udders
13	Peuch[47]	1889	France	1	1	100.0	Fed to pigs and rabbits
14	Galtier[21]	1889	France	1	0	0.0	
15	Ernst[18]	1890 to 1895	Boston, Mass.	36	15	41.7	Normal udders
16	Schmidt-Muhlheim[56]	1890		50	0	0.0	
17	Mac Fadyean and Woodhead[29]	1891	England	13	2	15.4	Normal udders
18	Bang[4]	1892	Denmark	60	6	10.0	Normal udders
19	Pearson[46]	1893	Philadelphia	1	1	100.0	
20	Smith and Schroeder[61]	1893	Washington, D. C.	6	2	33.3	
21	Law[28]	1894	Ithaca, N. Y.	3	3	100.0	Fed to calves
22	Schroeder[58]	1894	Washington, D. C.	16	1	6.3	
23	Hills and Rich[23]	1894	Vermont	Herd	Some	(?)	Fed to swine
24	Chester[10]	1895	Newark	1	0	0.0	Fed to guinea pigs
25	Obermüller[41]	1895	Berlin	(?)	(?)	(?)	38 percent of test animals positive
26	Gehrmann[22]	1895	Chicago	38	6	15.7	Guinea pig test
27	Ravenel[51]	1897	Pennsylvania	5	Some	(?)	Udders sound
28	Delépine[12]	1898	Liverpool	7	0	0.0	
29	Delépine[12]	1898	England	11	1	9.1	Udder tuberculous, *post mortem*
30	Bassett[7]	1899	Madison, Wis.	34	0	0.0	
31	Rabinowitsch and Kempner[49]	1899	Berlin	14	9	64.3	1 udder tuberculous, *post mortem*
32	Adami and Martin[1]	1899	Canada	10	6	60.0	Microscopic
33	Ascher[2]	1899	Königsberg	7	0	0.0	Sound udders
34	Rogers and Garnier[52]	1899	Italy	1	1	100.0	
35	Ostertag[42]	1899	Berlin	49	0	0.0	50 cows examined; one doubtful
36	Müller[36]	1899	Bolle, Germany	9	0	0.0	
37	Gehrmann and Evans[22]	1899	Chicago	41	15	39.0	Sound udders
38	Marshall[30]	1900	Lansing	50	1	2.0	
39	Douglas[17]	1900	England	7	0	0.0	Sound udders

*. Compiled from the experimental work of various investigators up to 1883.

†. Milk was fed to various test animals, among them two monkeys later found to be tuberculous; a result of doubtful significance.

TABLE 6.—*Continued*

No.	Author	Date	Place	Cows tested	Cows positive	Percent positive	Remark
40	Nonewitsch[40]	1900	Wilna, Russia	6	3	50.0	Six women *post mortem* examination
41	Ostertag[44]	1901	Berlin	18	0	0.0	
42	McWeeney[33]	1902	Ireland	15	0	0.0	All udders normal
43	Stenström[63]	1902	Hamra	50	0	0.0	Reacting cows
44	Mohler[34]	1903	Washington	56	12	21.4	
45	Prettner[48]	1904	Germany	3	0	0.0	Injected cultures into buffaloes
46	Russell[55]	1904	Madison, Wis.	19	1	5.3	Twenty-five examinations; six failed
47	Moussu[35]	1904	Paris	57	7	12.2	
48	Martel and Guerin[31]	1904	Germany	13	5	38 4	
49	Martel and Guerin[31]	1906	Germany	20	4	20.0	
50	Coquot and Cesari[11]	1908		1	0	0.0	
51	De Jong[14]	1908	Leiden	10	3	30.0	Milk taken with great care
52	Smit[60]	1909	Rotterdam	33	0	0.0	
53	Smit[60]	1909	Rotterdam	2	0	0.0	Natural tuberculosis, 33 tests
54	Smit[60]	1909	Rotterdam	2	2	100.0	Intravenous injection of bacilli
55	Royal Commission[53]	1909	London	4	2	50.0	Udders sound
	Total			748	131	17.9	

TABLE 7.—BACILLUS TUBERCULOSIS IN THE MILK OF TUBERCULOUS COWS WITH VISIBLY DISEASED UDDERS

No.	Author	Date	Place	Cows tested	Cows positive	Percent positive	Remark
1	May[32]	1883	Munich	1	1	100.0	
2	Bollinger[8]	1883	Germany	1	1	100.0	
3	Bang[3]	1884	Denmark	(?)	(?)	100.0	
4	Galtier[21]	1889	France	1	1	100.0	
5	Peuch[47]	1889	France	1	1	100.0	
6	Mac Fadyean[29] and Woodhead	1891	London	19	14	73.7	
7	Bang[4]	1892	Denmark	3	3	100.0	
8	Fiorentini[19]	1892	Italy	2	2	100.0	
9	Russell[54]	1894	Madison, Wis.	1	1	100.0	
10	Nocard and Leclainche[39]	1896	Paris	3	3	100.0	
11	Delépine[12]	1898	Manchester	6	6	100.0	
12	Rabinowitsch und Kempner[49]	1899	Berlin	1	1	100.0	
13	Douglas[17]	1900	England	(8)	(?)	(?)	48 samples, 34 positive
	Total			39	34	87.2	

cows were all capable of secreting tubercle bacilli in their milk. But since the milk from these cows was entirely negative to guinea pigs subcutaneously injected, he concluded that the danger which is attached to the milk of cows with closed tuberculosis absolutely need not be considered, and the chief source of danger is the cow with tuberculosis of the udder.

The investigations of Smit[60] were extensive and carefully done. He reviewed both the literature and methods of work at some length. His samples were taken with great care. The milk was drawn into a sterile flask thru a sterile rubber tube connected with a canula which was inserted into the opening in the teat. Sixty cubic centimeters of each sample were centrifuged and 5 cc. of an emulsion of the cream and sediment were injected into the muscle of the right hind leg of a guinea pig. The milk of 33 tuberculous cows upon which the diagnosis was to be confirmed by *post mortem* examinations was tested, as above described, and in none of the samples were tubercle bacilli found. The milk of two cows, one with open and the other with closed tuberculosis, was tested for the period of a month by both microscopic examination and animal inoculation, and no tubercle bacilli were found in any sample. A four-year-old cow, in full flow of milk, reacting to tuberculin, was injected intravenously with an emulsion of 50 mg. of pure culture of tubercle bacilli of the human type in 10 cc. of normal salt solution. Her milk, feces, and urine were tested for tubercle bacilli on each of three days before the injection. All these tests were negative. The same tests made after the injection showed the presence of tubercle bacilli in the feces samples on the first and third day. The urine sample of the first day only contained tubercle bacilli. Eighteen samples of milk were taken during the month with which 23 guinea pigs were inoculated. The milk samples taken on the second day after the injection contained tubercle bacilli, all others were negative. In a similar manner a non-tuberculous cow was injected with 25 cc. of a thick emulsion of a tuberculous lung from another cow and her milk was tested for tubercle bacilli. The control tests before the injection were all negative. The tests of the urine following the injection of lung emulsion were negative; the tests of the feces samples were lost; the tests of the milk on the first, second, and third days after the injection showed the presence of tubercle bacilli in each sample. The tests of all other samples during the month were negative.

INVESTIGATORS REPORTING POSITIVE RESULTS

Of the authors reporting positive results on testing milk from tuberculous cows with sound udders may be mentioned Bang[4,5], Smith and Schroeder[61], Ravenel[50], Rabinowitsch and Kempner[49], Mohler[34], Moussu[35] De Jong[14], The Royal Commis-

sion on Tuberculosis, England.[53] These investigators have made just as careful tests as those just reported who found negative results. Special mention will be made here of only the last two.

De Jong had special opportunity to investigate this question, as he was an officer in the official slaughtering house of Leiden, Holland. Cows that were condemned because of the tuberculin test became the property of the State. So De Jong was able to select just such cows as he chose and obtain samples immediately before slaughtering. This he did and selected cows with closed tuberculosis as determined by testing the saliva, trachial or bronchial mucus and genital secretions. The samples were very carefully taken. Before milking, the udder, the teats, and the whole hind parts of the cow were carefully washed with warm soap solution and especial attention paid to the opening in the teats. After the washing with the soapwater the hind parts of the cow were very carefully washed with 3 percent boracic acid, as were also the hands of the milker. The first part of the milk was discarded and only the last part drawn into a sterile flask. Forty cubic centimeters of this milk were centrifuged at 3200 revolutions per minute. The cream and sediment were mixed with a part of the skim-milk and 10 cc. of this were injected into the peritoneal cavity of one guinea pig and 5 cc. under the skin of the hind leg of another. He tested ten cows and found tubercle bacilli in the milk of three.

The Royal Commission on Tuberculosis has made very thoro tests upon five cows, three of which, B, C, and F, showed obvious signs of tuberculosis, and two of which, D and E, showed no visible signs. None of the five cows showed any sign of udder tuberculosis during life. All the cows were subsequently slaughtered and the udders were carefully examined for tubercles and tubercle bacilli. There were none found except in one case, cow F, in which one quarter showed four small nodules which could not possibly have been detected during life. For the autopsy notes, see Table 8. Strictest precautions were taken in the collection of the samples. A pressure tube was connected to a metal catheter which was inserted into the milk sinus of the teat. The teat and the udder were thoroly cleaned. They were washed with a solution of mercuric chloride and the opening of the teat carefully cleansed so that no tubercle bacilli could be pushed back into the sinus. The milk from each quarter was collected separately and was inoculated into guinea pigs and rabbits. Two guinea pigs were inoculated intraperitoneally from each quarter. Each animal received a dose of ten cubic centimeters of uncentrifuged milk plus the deposit of 20 cc. of centrifuged milk, or sometimes 6 cc. plus the deposit of 24 cc. of centrifuged milk. Three of the five cows

TABLE 8.—TUBERCLE BACILLI IN FECES AND IN MILK OF SIX NATURALLY TUBERCULOUS COWS

	Lesions in the cow at autopsy				Guinea pigs inoculated with feces				G. pigs fed with feces		Swine fed with feces		Guinea pigs inoculated with milk			
Cow	Lung	Intestine	Udder	Uterus	Total fed	Died early	Healthy	Tuberculous	Total fed	Tuberculous	Total fed	Tuberculous	Total inoculated	Died early	Healthy	Tuberculous
Obviously Tuberculous Cows																
B	++	++	0	++	25	8	1	16	22	0	4[5]	4	56[3]	0	54[4]	1
C	+	+	0	++	30	13	0	17	14	1	1	1	28	0	19	9
F	+	+	+	++	7	4	0	3	..	..	..	..	32	11	0	21
Cows not Obviously Diseased																
A	+	+	0	+	41[1]	1	40	0	..	..	4	1	..	..	..	..
D	+	0	0	+	14	0	8	6	..	..	2	0	60	0	60	0
F	+	0	0	+	18	3	15	0	..	..	2	0	40[2]	0	40	0

\+ Tubercles present.
++ Extensive tuberculosis.
1. Some tested by feeding.
2. Four of these forty were rabbits.
3. One guinea pig became tuberculous, but was rejected because the omentum was free from tubercles.
4. Rabbits were used in part; three of these became tuberculous.
5. Milk from Cow B was also fed to three swine; none of them became tuberculous.

(see Table 8) tested were giving milk which contained *Bacillus tuberculosis*. In one of these, cow F, some small tubercles in one quarter of the udder were discovered at autopsy. The milk of cows B and C as shown by guinea pig inoculations contained tubercle bacilli. The milk was withdrawn from the udder by catheterisation, and post mortem examination of the udders revealed no microscopical evidence of tuberculosis. Small pieces from the udder were examined histologically and found to be normal. The milk of the other two cows, D and E, did not give rise to tuberculosis in any instance. Altogether, one hundred test animals were inoculated with the milk of these two cows.

EXPERIMENTAL

SAMPLES EXAMINED

Milk has been examined for *Bacillus tuberculosis* from three different sources:

1. Milk from thirty-five tuberculous cows of the University of Illinois Experiment Station, taken collectively.

2. Milk from three individual cows of the tuberculous herd selected as the most advanced cases in the herd.

3. Milk from the individual teats of six tuberculous cows that were soon to be slaughtered and examined *post mortem*.

MILK FROM THE TUBERCULOUS HERD

Eleven samples of mixed milk from thirty-five tuberculous cows of the herd of the University of Illinois Agricultural Experiment Station were tested. The samples were taken at the morning milking time from the large milk cans after thoroly mixing the milk. The milking of the cows in the tuberculous herd is done with considerably more care than is usual on the farm. The method of handling the sample at the laboratory has been described under Market Milk, general method. The results are given in Table 9. Ten of the eleven samples completed the test. Sample No. 5 was lost, all the guinea pigs dying early. Three of the

TABLE 9.—EXAMINATION OF MILK FROM THE UNIVERSITY TUBERCULOUS HERD

Sample number	Guinea pig number	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Tuberculosis	Remark
1	9	7-9-'08	Killed	63	Normal	0	
	10	"	Died	2	Acute infection		
	11	"	Killed	63	Normal	0	
2	21	7-24-'08	Died	7	Acute infection		
	22	"	Died	12	Acute infection		
	23	"	Killed	56	Normal	0	
3	30	8-6-'08	Killed	57	Normal	0	
	31	"	Killed	57	Normal	0	
	32	"	Killed	57	Normal	0	
4	56	8-11-'08	Killed	57	Normal	0	
	57	"	Killed	57	Normal	0	
	58	"	Killed	57	Normal	0	
5	59	8-13-'08	Died	1	Acute infection		
	60	"	Died	1	Acute infection		
	61	"	Died	1	Acute infection		
6	75	8-18-'08	Killed	60	Normal	0	
	76	"	Killed	60	Normal	0	
	77	"	Killed	60	Normal	0	
7	87	8-20-'08	Died	49	No tubercles	0	
	88	"	Killed	61	Normal	0	
	89	"	Died	51	No tubercles, large tumor on intestines	0	
8	93	8-25-'08	Killed	63	Normal	0	
	94	"	Killed	63	Normal	0	
	95	"	Killed	63	Normal	0	
9	105	8-27-'08	Killed	62	Normal	0	
	106	"	Killed	62	Normal	0	
	107	"	Died	46	No tubercles	0	
10	113	9-1-'08	Died	41	No tubercles		
	114	"	Died	61	No tubercles		
	115	"	Died	63	No tubercles		
11	125	9-3-'08	Died	58	No tubercles		
	126	"	Died	59	No tubercles		
	127	"	Died	60	No tubercles		

Summary: Ten samples completing the test; none with tubercle bacilli.

other thirty guinea pigs used in testing these ten samples died too early to determine the presence or absence of tubercle bacilli. None of the remaining 27 guinea pigs became tuberculous.

MILK FROM THE THREE MOST ADVANCED CASES

Six samples of milk from three individual cows of the University tuberculous herd were tested for tubercle bacilli. These cows were selected as being the most advanced cases in the herd. After the usual grooming of the cow and washing of the udder, about one-third of the milk was drawn from each teat; the udder was washed again with five percent carbolic acid and then with boiled water and dried with sterile cotton. The sample was milked directly into the sterile flask, an equal part from each quarter of the udder. At autopsy the udders of all these cows were found to be free from lesions of tuberculosis. Two of the animals, Nos. 28 and 53, were slaughtered at a local abattoir and personally examined *post mortem*, the other one, No. 13, was slaughtered in Chicago under federal inspection. (See Table 19.)

The results of the tests are given in Table 10. None of the eighteen guinea pigs, inoculated with the milk, became tuberculous.

MILK FROM SEGREGATED QUARTERS OF THE UDDER

Milk from the segregated quarters of six cows from the tuberculous herd was examined with more than usual care for tubercle bacilli. These were six cows which were to be slaughtered, thus giving us an opportunity to make a careful autopsy in a very short time after taking the sam-

TABLE 10.—EXAMINATION OF MILK FROM THREE INDIVIDUAL COWS OF THE UNIVERSITY TUBERCULOUS HERD

Sample	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Tuberculosis	Remark
Cow	66	8-15-'08	Killed	56	Normal	0	
13	67	8-15-'08	Killed	56	Normal	0	
(1)	68	8-15-'08	Killed	56	Normal	0	
Cow	96	8-26-'08	Killed	62	Normal	0	
13	97	8-26-'08	Killed	62	Normal	0	
(2)	98	8-26-'08	Killed	62	Normal	0	
Cow	69	8-15-'08	Killed	55	Normal	0	
28	70	8-15-'08	Killed	55	Normal	0	
(1)	71	8-15-'08	Killed	55	Normal	0	
Cow	99	8-26-'08	Killed	63	Normal	0	
28	100	8-26-'08	Died	13	Acute infection	0	
(2)	101	8-26-'08	Killed	63	Normal	0	
Cow	72	8-15-'08	Killed	55	Normal	0	
53	73	8-15-'08	Killed	55	Normal	0	
(1)	74	8-15-'08	Killed	55	Normal	0	
Cow	102	8-26-'08	Killed	63	Normal	0	
53	103	8-26-'08	Killed	63	Normal	0	
(2)	104	8-26-'08	Killed	63	Normal	0	

ples of milk. In this way it was hoped to correlate our results more accurately with the autopsy findings, and if possible to get some data concerning the secretion of tubercle bacilli in the milk from cows with sound udders. The samples from the segregated quarters were taken with aseptic technic. The apparatus shown in Fig. 3 was used. It consists of a milking tube (canula) attached to a flask with rubber tubing. The milking tube is protected from contamination by the test tube. The stopper of the flask is covered with cotton: this is necessary for tho the flask is closed tightly with a rubber stopper water may be drawn thru by

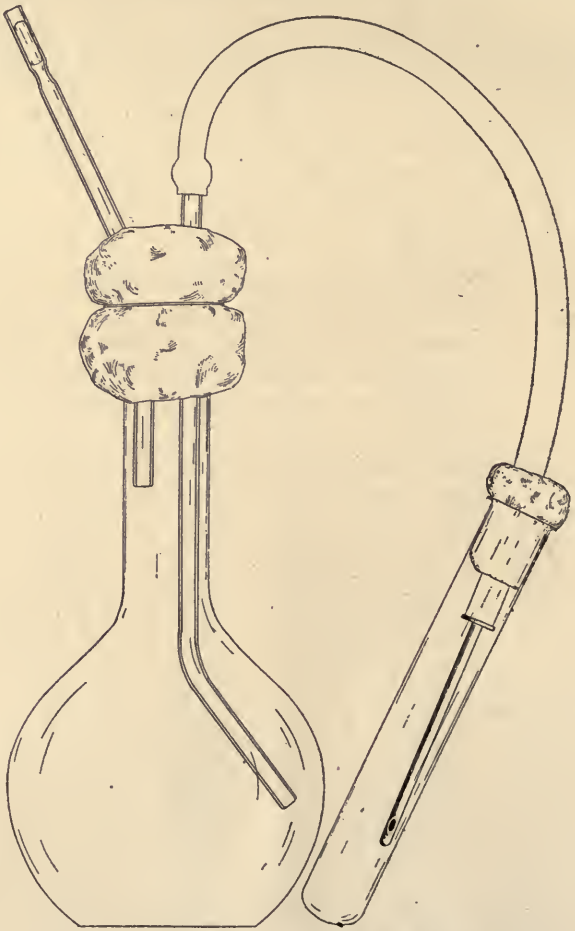

FIG. 3. FLASK FOR COLLECTING MILK SAMPLES.

capillarity and thus be a source of contamination. The whole instrument was sterilized in the autoclave at fifteen pounds pressure for fifteen minutes.

Before taking the sample the cows were groomed and the udders and hind parts of the cow thoroly washed with soap and water. One-third of the milk was drawn from each quarter, and then the udder was again washed with five percent carbolic acid and then with boiled water and dried with sterile cotton. The openings of the teats were more thoroly cleansed by picking out with a sterilized tooth pick any dirt found there in order that no feces or other dirt should be pushed up into the sinus of the teat by the milk tube. Again the teats were washed with five percent carbolic acid and boiled water. The hands of the operator were also washed in a similar way. The test tube protecting the milk canula was next removed and the canula immediately inserted into the opening of the teat. The milk flowed of its own accord as a rule; at times, a little pressure of the hand upon the udder was necessary.

A number of these samples were tested by counts of colonies upon gelatin plates inoculated with 0.5 cc. and 0.25 cc. of milk. Tho the counts upon gelatin plates after 48 hours at a temperature of 20° C. may not reveal all of the bacteria which have been found to be present in milk, such as milk streptococci, yet the gelatin plate gave us the information desired for our purpose, namely, to determine if the samples were being contaminated by feces or other dirt from the outside.

A number of these samples were tested, of which the results from the samples of two cows taken at different times are given. Samples of milk from cow 54, tested on September 17, 1909, gave the following numbers of bacteria per cubic centimeter on the gelatin plates:

	Number of bacteria.
1. From right forequarter	0 per cc.
2. From right hindquarter	5 per cc.
3. From left forequarter	0 per cc.
4. From left hindquarter	4 per cc.

Samples from cow 58, September 14, 1909:

	Number of bacteria.
1. From right forequarter	0 per cc.
2. From right hindquarter	0 per cc.
3. From left forequarter	0 per cc.
4. From left hindquarter	320 per cc.

It is probable that some little dirt particles in some way entered the last sample. The counts of most of the samples were similar to those from cow 54. We were assured that on the whole we were not getting any considerable contamination from outside the udder.

The results are given in Table 11. The milk from each cow was tested twice. For the first testing, two guinea pigs were used for each of the four samples taken or eight guinea pigs for each cow. For the second testing, on account of a lack of guinea pigs at the time, cows No. 54 and No. 52 were tested with one guinea pig each per sample. Cows No. 37, No. 57, No. 58, and No. 10 were tested by drawing the milk aseptically from the four quarters into one flask, and this composite sample was tested by inoculation into two guinea pigs. Three to four cubic centimeters of the cream and sediment of 80 cc. of the centrifuged milk samples were inoculated into each of the two guinea pigs. In none of the samples were tubercle bacilli detected. Microscopical tests were not made upon any of the samples. (For autopsy records of these cows see Table 19.)

TABLE 11.—EXAMINATION OF MILK FROM EACH TEAT OF SIX TUBERCULOUS COWS

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days since inoculation	Autopsy	Tuberculosis	Remark
57	1 R. F.	549	9-6-'09	Killed	44	Normal	0	
		550	9-6-'09	Killed	44	Normal	0	
	2 R. H.	551	9-6-'09	Killed	44	Normal	0	
		552	9-6-'09	Killed	44	Normal	0	
	3 L. F.	553	9-6-'09	Killed	44	Normal	0	
		554	9-6-'09	Killed	44	Normal	0	
	4 L. H.	555	9-6-'09	Killed	44	Normal	0	
		556	9-6-'09	Killed	44	Normal	0	
37	5 R. F.	557	9-8-'09	Killed	42	Normal	0	
		558	9-8-'09	Killed	42	Normal	0	
	6 R. H.	559	9-8-'09	Killed	42	Normal	0	
		560	9-8-'09	Killed	42	Normal	0	
	L. F.	561	9-8-'09	Killed	41	Normal	0	
		562	9-8-'09	Killed	41	Normal	0	
	8 L. H.	563	9-8-'09	Killed	41	Normal	0	
		564	9-8-'09	Killed	41	Normal	0	
58	9 R. F.	565	9-9-'09	Killed	40	Normal	0	
		566	9-9-'09	Killed	40	Normal	0	
	10 R. H.	567	9-9-'09	Killed	40	Normal	0	
		568	9-9-'09	Killed	40	Normal	0	
	11 L. F.	569	9-9-'09	Killed	40	Normal	0	
		570	9-9-'09	Killed	40	Normal	0	
	12 L. H.	571	9-9-'09	Killed	40	Normal	0	
		572	9-9-'09	Killed	40	Normal	0	
10	13 R. F.	575	9-10-'09	Killed	61	Normal	0	
		576	9-10-'09	Killed	61	Normal	0	
	14 R. H.	577	9-10-'09	Killed	61	Normal	0	
		578	9-10-'09	Killed	61	Normal	0	
	15 L. F.	579	9-10-'09	Killed	61	Normal	0	
		580	9-10-'09	Killed	61	Normal	0	
	16 L. H.	581	9-10-'09	Killed	61	Normal	0	
		582	9-10-'09	Killed	61	Normal	0	

TABLE 11.—*Continued*

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days since inoculation	Autopsy	Tuberculosis	Remark
52	17 R. F.	588	9-14-'09	Killed	59	Normal	0	
		589	9-14-'09	Killed	59	Normal	0	
	18 R. H.	590	9-14-'09	Killed	59	Normal	0	
		591	9-14-'09	Killed	59	Normal	0	
	19 L. F.	592	9-14-'09	Killed	59	Normal	0	
		593	9-14-'09	Killed	59	No Tubercles[1]	0	
	20 L. H.	594	9-14-'09	Killed	59	Normal	0	
		595	9-14-'09	Died	5 min	Note[2]	0	
54	21 R. F.	600	9-15-'09	Killed	58	Normal	0	
		601	9-15-'09	Killed	58	Normal	0	
	22 R. H.	602	9-15-'09	Killed	58	Normal	0	
		603	9-15-'09	Killed	58	Normal	0	
	2 L. F.	604	9-15-'09	Killed	59	Normal	0	
		605	9-15-'09	Killed	59	Normal	0	
	24 L. H.	606	9-15-'09	Killed	59	Normal	0	
		607	9-15-'09	Killed	59	Normal	0	
	25 R. F.	614	9-17-'09	Killed	61	Normal	0	
	26 L. H.	615	9-17-'09	Killed	61	Normal	0	
	27 L. F.	616	9-17-'09	Killed	61	No Tubercles[3]	0	
	28 L. H.	617	9-17-'09	Killed	61	Normal	0	
52	29 R. F.	626	9-18-'09	Killed	60	Normal	0	
	30 R. H.	627	9-18-'09	Killed	60	Normal	0	
	31 L. F.	628	9-18-'09	Killed	60	Normal	0	
	32 L. H.	629	9-18-'09	Killed	60	Normal	0	
37	33 (all)	634	9-22-'09	Killed	56	Normal	0	
		635	9-22-'09	Killed	56	Normal	0	
57	34 (all)	636	9-22-'09	Killed	56	Normal	0	
		637	9-22-'09	Killed	56	No Tubercles[4]	0	
58	35 (all)	638	9-22-'09	Killed	56	Normal	0	
		639	9-22-'09	Killed	56	Normal	0	
10	36 (all)	632	9-22-'09	Killed	56	Normal	0	
		633	9-22-'09	Killed	56	Normal	0	

1. Guinea pig 593 had some small white lesions in the liver which were not tuberculous.
2. Guinea pig 595 died five minutes after injection.
3. Guinea pig 616 had an ulcer at the point of inoculation; a microscopic examination showed micrococci and streptococci; no tubercle bacilli.
4. Guinea pig 637 showed a small abscess in the lung; tubercle bacilli were not found.

SUMMARY AND DISCUSSION

Whether tubercle bacilli pass thru the sound udder of a tuberculous cow has been a problem under consideration now for considerably more than a quarter of a century. That bacteria pass thru the healthy epithelium of animals without forming lesions has been shown by a number of investigators. Dobroklonski[16] in 1890 found that tubercle bacilli, after only a short contact, will penetrate the intestinal wall in the absence of any demonstrable lesions.

In 1895 Nocard[39] observed that blood drawn from horses under the strictest precautions would often show contamination if taken at certain periods of digestion, while at other times no such contamination would occur. In the same year two of Nocard's pupils, Desoubry and Porcher[15], continued these researches and found that, in dogs, during the digestion of fat, large numbers of bacteria were carried thru the intestinal wall and could be detected in the chyle by means of plate cultures. If the dogs were fed material free from fat very few or even no bacteria were found in the chyle. In 1902 Nicolas and Decos[37] showed that tubercle bacilli will pass thru the healthy intestines of dogs during digestion. In the same year Ravenel[51] carried out a more extended experiment. Ten healthy dogs were selected, purged with castor oil and fasted for 24 hours. They were then given thru a stomach tube a single dose of an emulsion, made of equal parts of melted butter and warm water, containing a large number of tubercle bacilli. Three and one-half to four hours later the dogs were killed and as much chyle as possible collected, together with the mesenteric lymph glands. With this material guinea pigs were inoculated intraperitoneally. Microscopic examinations were also made. The entire intestine was then carefully examined, microscopic sections made; in no instance were lesions detected. The guinea pigs inoculated with the material from eight of the ten dogs became tuberculous. The author concluded that the tubercle bacilli pass thru the intestinal wall without forming lesions.

It is well known that in such diseases as anthrax and foot and mouth disease of cattle the infectious agent passes into the milk. Schreiber and Neuman[57], after injecting repeated doses of a culture of *Bacillus rhusiopathiae* or swine erysipelas intravenously, found that these organisms did not pass into the milk thru the healthy udder of the cow.

In chronic localized tuberculosis of milk cows the tubercle bacilli are not usually found in milk that is drawn with extreme care so as not to be contaminated from the outside. Note the works of Ostertag[42], Stenström[63], Bang and Nocard[6], and Smit[60].

The work of Ostertag (page 344) has been discussed. He has followed up this work with some additional evidence and numerous discussions and papers. In April, 1908, Ostertag[44] reviewed critically the literature of those investigators who have found positive results. In general, he states that these investigators had not been careful enough in taking their samples to avoid contamination of the milk from the outside. He says some of the investigators had not taken their own samples (Mohler, Rabinowitsch and Kempner) and that others had taken the samples in

open vessels in the presence of cows with open tuberculosis and in stables where such cows were kept. Therefore Ostertag still holds that cows evidently reacting to tuberculin but with sound udders produce milk free from tubercle bacilli; but that in cows with badly generalized tuberculosis the liability to external contamination is very great.

In examining the literature we have found that, as a rule, where mention is made of autopsy records, cows giving milk free from tubercle bacilli were suffering from localized rather than generalized tuberculosis. On the other hand, cows having chronic generalized tuberculosis, but with sound udders, may give milk containing tubercle bacilli. Such positive findings have been reported by Rabinowitsch and Kempner[49] 1899, Mohler[34] 1903, Moussu[35] 1904, Martel and Guerin[31] 1904, and 1906, Bang and Nocard[6] 1908, de Jong[14] 1908, and the Royal Tuberculosis Commission[53] 1909. The work of these investigators has been done with extreme care and with the knowledge that their reports would be criticised and scrutinized very carefully by other workers. An examination of the autopsy records shows that in most cases the cows furnishing the milk for these positive findings had extensive generalized tuberculosis. This fact may be especially noted in the work of Mohler, Bang and Nocard, and that of the Royal Tuberculosis Commission. The autopsy records and direct statements of these authors show that every cow furnishing the milk containing tubercle bacilli was severely affected with generalized tuberculosis.

In those experiments in which pure cultures of tubercle bacilli have been injected into the blood stream and the milk subsequently examined, conflicting results have been obtained by different authors. Ostertag,[45] Prettner,[48] Coquot and Cesari[11] have found negative results; while on the other hand Smit[60] has found tubercle bacilli present in samples of milk from each of the two cows that he injected with tubercle bacilli.

The investigations of Calmette and Guerin[9] show that the livers of rabbits, which have been injected intravenously with pure culture of tubercle bacilli, excrete tubercle bacilli in the bile. Since it is quite evident that the normal liver can excrete tubercle bacilli in the bile, we may expect that in cows with highly generalized tuberculosis, tubercle bacilli may be excreted in the milk from a sound udder. The evidence points strongly in this direction. It is probably in only the very bad cases, when the tubercle bacilli are free in the circulating blood, that this occurs.

In another section of this bulletin, there has been discussed at some length the possible presence of tubercle bacilli in the circulating blood. The importance of this is seen here; for if the

circulating blood contains tubercle bacilli it will not only be dangerous to consume meat from such animals, but the milk as well.

CONCLUSIONS

1. Those investigators finding positive results and those finding negative results in testing milk of tuberculous cows with sound udders are about equal in number as shown by the literature cited in Tables 6 and 7.

2. Tho the question is yet unsettled whether tuberculous cows with sound udders secrete tubercle bacilli in their milk, the facts so far accumulated point to these conclusions:

(a). Tubercle bacilli are rarely found in the milk of tuberculous cows with sound udders, especially if the infection is localized.

(b). When a cow has extensive generalized tuberculosis or when the tubercle bacilli are *free in the blood* (as, for example, after injecting a pure culture of tubercle bacilli into a vein), *at such times tubercle bacilli may be secreted along with the milk.*

3. We have tested for tubercle bacilli a total of forty-seven samples of milk from ten tuberculous cows. All the cows at autopsy showed normal udders except No. 57. This one showed extensive induration in the right forequarter of the udder, but this change was not tuberculous in nature. In none of the forty-seven samples were any tubercle bacilli found, and at autopsy none of the ten cows was found to be very extensively tuberculous.

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EXAMINATION OF FECES FOR TUBERCLE BACILLI.

INTRODUCTION.

Schroeder and Cotton[9] first called attention to the frequency with which the feces of cattle contain living tubercle bacilli, and the possibility of their introduction into milk in ths way. *"We have seen, also, from the work of the United States Public Health and Marine-Hospital Service that among* 172 *samples of city milk examined* 121 *or* 70 *percent contained a sediment after standing a few hours in the original containers, and that the sediment consisted in part of cow feces".* (Schroeder[7] pages 14-15.) Truman[12] examined 232 samples of milk collected in the city of Chicago. Of these, 158 samples or 68 percent contained visible sediment. Of 212 samples of milk collected in other Illinois cities of over 10,000 inhabitants he found 88 percent containing visible sediment. These facts indicate the danger to man from the use of milk contaminated with manure of tuberculous cows, and because of the frequency with which fecal material from cows is present in market milk this phase of the subject seems worthy of careful study. Furthermore, the recognition of the importance of tubercle bacilli in the manure is a rather recent development in the study of bovine tuberculosis. For these reasons it is considered in some detail.

LITERATURE

Schroeder and Mohler[10] first proved that tubercle bacilli will pass thru the alimentary tract of cattle and pass out in their feces alive and virulent for hogs. They also performed several

experiments by adding to milk, feces from cows known to be passing tubercle bacilli, and then inoculating guinea pigs with this soiled milk. In this way they were able to produce tuberculosis in the guinea pigs. They claim that the chief source of danger lies in the contamination of the milk with feces which may contain tubercle bacilli. Schroeder and Cotton[6] examined by microscopial tests seven natural cases of tuberculosis in cattle, and found tubercle bacilli in the feces in five or 71.43 percent of the cases. They consider the microscopical test a reliable means of determining the bacilli in cow feces. They say *"At first it was believed that the bacilli in the preparations belonged in whole or in part to the latter group (harmless acid-fast bacteria) but this view was not tenable after it was discovered that similar bacilli could not be found in material from non-tuberculous cattle."* (Schroeder and Cotton,[6] p. 9.) Inoculation experiments were made with the feces of the five cows, into guinea pigs, with the result that three of the five were shown to be passing virulent tubercle bacilli. They consider feces to be the most dangerous factor in the dissemination of bovine tubercle bacilli. *"In this respect feces must be regarded as having a place with cattle similar to that commonly accorded to sputa with tuberculous persons."* (Schroeder and Cotton,[6] p. 22.) They conclude that every cow known to be affected with tuberculosis must be regarded as positively dangerous, since we are unacquainted with any means by which it can be determined when tuberculous cattle or their feces become dangerous to the health of persons or animals.

Schroeder[7] says that practically no tuberculous herd is very long free from a dangerously tuberculous animal, and since we cannot tell when a tuberculous cow becomes dangerous we are forced to assume for practical purposes that every tuberculous cow is dangerous from the moment she is affected with tuberculosis. She may be dangerous long before the disease is indicated by her physical appearance. He says that about forty percent of all cows reacting to tuberculin are passing virulent tubercle bacilli in the feces, this being the commonest mode of expelling these germs from a cow's body. In a later communication he further emphasizes the danger to man from this source. He collected twelve tuberculous cows from several dairy herds and kept them under observation for two years. They were in excellent general condition and had no visible symptoms of the disease. During the first two months microscopial examinations showed that five or 41⅔ percent were expelling tubercle bacilli; eighteen months later ten or 83⅓ percent were expelling these germs in the feces. He says that ordinary market milk always contains sediment consisting of cow dung. The micro-organisms are very easily de-

tached and washed from the dung into the milk. Such milk inoculated into guinea pigs produces tuberculosis. Tubercle bacilli are found in the separated cream as well as in the sediment. Butter made from such cream also contains them in a condition virulent to guinea pigs. In ordinary salted butter they may remain alive at least 160 days. In conclusion Schroeder[8] says that tuberculous cows frequently expel tubercle bacilli per rectum. These germs may come from the lungs and not necessarily from the intestinal mucosa. They are evenly distributed in the mass of the feces and expelled in large numbers; easily enter milk, butter and ice-cream and may thus be transmitted to human beings. He concludes further that since Mohler and Washburn have converted the bovine type of tubercle bacillus into the human type by passage thru cats (which work he accepts as trustworthy), this may occur in the human body as readily, and it is therefore imperatively necessary for the protection of public health that all milk should be obtained from cows certainly free from tuberculosis, and from cows stabled, milked, and pastured in environments free from tuberculous infection, or else the dairy products should be pasteurized or sterilized before they are used as food in any form.

Reynolds and Beebe[5] have also examined the feces of tuberculous cattle. They had for their material feces of 45 cattle which had reacted to tuberculin. These were, for the most part, pure bred cattle. The majority of them had reacted to tuberculin two years and a part several years before the experiment was made. The manure of sixteen of these was tested three times; that of one, five times; of two, four times; of fourteen, twice, and of seven, once. Of the entire herd, only one animal was excreting virulent tubercle bacilli in her feces. Her nasal secretions also contained *Bacillus tuberculosis.* The feces were collected with a rectal spoon. The hind parts of the cow were previously disinfected in most cases. Two grams of feces were rubbed up in sterile water. At first 5 cc. of this emulsion were injected subcutaneously; this killed all the guinea pigs. Later, 0.5 cc. of the emulsion was used and this worked fairly well. Microscopic test for the presence of tubercle bacilli in manure is not reliable according to these authors. In the single case in which the animal inoculation proved the presence of the tubercle bacilli they were unable to detect these germs in the feces by the use of the microscope. They also consider it impossible to distinguish with certainty by microscopic examination alone, between tubercle bacilli and other acid-fast bacteria which may be present in the feces of the cow.

The Royal Commission on Tuberculosis[6] tested the samples

of feces from six naturally tuberculous cattle, of which three showed physical signs of tuberculosis. For the purpose of the experiments, a considerable quantity of fecal matter was required. The action of the rectum was stimulated by injecting air into it thru a sterile glass tube and the feces were received directly into a sterilized pail applied to the margin of the anal orifice. This was necessary to prevent contamination from the vagina since three of the cows tested had extensive tuberculosis of the uterus and one, at least, was excreting purulent material from the vaginal outlet. A portion of the feces was rubbed up in a mortar with sufficient normal salt solution to form an emulsion that could be pressed thru muslin. It was found that 0.05 cc. of this emulsion injected intraperitoneally did not kill the test animals with acute peritonitis. Feces were fed also to guinea pigs and swine. The swine were young animals, about eleven weeks old, all belonging to three litters, raised upon the same farm. They were all tested with tuberculin and found free from tuberculosis before they were used in the experiments. One pig from each of the two litters was kept as a control and when killed at the end of the experiment was quite normal. The feces were fed to the swine in previously sterilized milk, each animal being fed for about one week, receiving in this time several pounds of moist feces. Some guinea pigs were fed the tuberculous feces in sterilized milk, and others received a single dose administered by a pipette. The dose given was one cubic centimeter of the emulsion used for inoculation, a quantity fifty times that employed for intraperitoneal injection. Except those guinea pigs that died early and were thus thrown out of the test, every guinea pig save one inoculated with feces from the three obviously tuberculous cows become tuberculous. Every one of the five swine fed the feces from two of these cows became tuberculous. The feces of the other cow were not fed to swine. From cow D, one of the cows that showed no outward signs of tuberculosis, six of the fourteen guinea pigs inoculated became tuberculous. The 41 guinea pigs inoculated with feces from cow A, and 18 guinea pigs inoculated with feces of cow E remained free from tuberculosis. Four swine were fed with the feces of cow A and one of them became tuberculous. Two swine were fed with the feces from cow D, and two with the feces of cow E. These animals remained free from tuberculosis. The feeding of 36 guinea pigs with the feces of cows B and C produced only one tuberculous guinea pig. This one was fed with the feces of cow C. The feces from these cows, B and C, were shown by inoculation into guinea pigs and by feeding swine to be very virulent. It thus appears that feeding suspected material to guinea pigs is not a reliable test for the presence of

tubercle bacilli. Their results show that five of the six naturally tuberculous cows were passing virulent tubercle bacilli in their feces as shown by inoculation and feeding tests. Two of these cows, A and D, were apparently in excellent health. Cow A, upon *post mortem* examination, was found to have tubercles in the lungs and in the small intestine; cow D had a bad lung, with pus in the bronchial tubes. For a tabulation of the results see Table 8, page 349, under literature of milk investigation; also the autopsy records of these cows, page 388.

Peters and Emerson[4] consider the microscope alone of little value in the examination of feces for tubercle bacilli, because in feces similar bacilli are met with, which stain in the same way and cannot be distinguished morphologically from the tubercle bacilli. Moreover, the real tubercle bacilli may be present in such small numbers that a microscopic examination would be inadequate to disclose them, as was shown by Reynolds and Beebe. Guinea pig inoculations therefore must be resorted to; and not only that, but the characteristic lesions of tuberculosis must be produced in the animals before the presence of tubercle bacilli can be accepted as proven. In their experiments these authors used forty-one cows. The majority of the cows were in excellent physical condition. In all cases, the tuberculin test had been applied only a few weeks before making the examinations. The feces were taken with a rectal spoon. Microscopical examinations were made for acid-fast organisms, and when such were found, guinea pigs were inoculated. For these inoculations the manure was suspended in water and 0.5 to 1 cc. of the suspension injected. The guinea pigs died of septicemia in only a few instances. Feces from twenty-two cows were tested in this way and, of these, three were shown to be passing virulent tubercle bacilli. Two of these three were in good physical condition, and from their appearance the disease would not have been suspected. The third was thin but signs of the disease were not marked.

Calmette and Guerin[2] injected pure cultures of tubercle bacilli into the ear vein of several rabbits. On each of seven days after these injections a rabbit was killed and immediately the bile was removed, centrifuged, and the sediment injected into four guinea pigs. Forty-five days later all the guinea pigs were killed and examined, with the following results: Two of the four guinea pigs inoculated with the bile sediment from the rabbit killed on the third day after the injection were tuberculous. One of the four guinea pigs inoculated with bile sediment from the rabbit killed on the fourth day after its injection was tuberculous. All of the eight guinea pigs inoculated with bile sediment from the fifth and

sixth day rabbits were tuberculous, and three of the four guinea pigs inoculated with bile sediment from the seventh day rabbit were tuberculous. It is therefore evident that tubercle bacilli may be excreted by the liver and pass with the bile into the intestine.

EXPERIMENTAL

COLLECTION OF THE SAMPLES For the first series of examinations of the dairy herd the samples were all taken directly from the rectum of the cow with a sterilized bone spoon by one of us (Briscoe) and transferred to a sterile four ounce glass-stoppered bottle. This was usually done while the cow was in the act of defecating but at times the sample was taken by pushing the bone spoon several inches into the rectum, revolving and then withdrawing it. By repeating this operation once or twice eight to ten grams of feces were obtained. The spoon was washed in five percent carbolic acid and dried with a sterile cloth before taking each sample. The sample of feces for the second series, and all samples since then, have been taken by the method recommended by the Royal Commission of England, viz., by pumping air into the rectum and thus stimulating the cow to void. This method has worked exceedingly well. It was often possible in this way to cause a cow to void again immediately after a natural defecation. Thick walled sterile glass tubes ten inches long with edges well rounded off in the flame were attached by rubber tubing to a rubber bulb air pump. The glass tubes were kept in a sterile tin case and one used for each cow. The tube was inserted into the rectum for five or six inches and air pumped in until the cow was stimulated to void. This required only a few minutes and very rarely failed. The feces were caught in a one-half gallon sterile tin pail provided with a tight fitting cover. The pail was opened only while collecting the sample. It was held close up to the anus in order to avoid getting any material from the vagina. About five hundred grams of feces were collected for each sample.

MAKING THE SUSPENSION The sample was mixed with a sterile glass rod and exactly one gram of it transferred to the inside of the neck of a sterile fifty cubic centimeter, glass-stoppered, measuring flask.* This was moistened with sterile 0.8 percent salt solution, and rubbed to an emulsion in the neck of the flask with a sterile glass rod. The flask was then filled one-third full by the addition of salt solution

*This method of preparing a suspension of feces has been described by MacNeal, Latzer, and Kerr.[3]

and thoroly shaken, inverted, and the sediment allowed to fall back into the neck. By gradually tipping back the flask, the sediment was left in the neck where it was again rubbed up with the glass rod. This was repeated until the bacteria of the feces were brought thoroly into suspension, and the pieces of straw appeared quite clean. The flask was now filled to the mark and the contents thoroly mixed. This gave a two percent suspension of feces.

CENTRIFUGATION

Forty cubic centimeters of this suspension were transferred by means of a sterile Pasteur bulb pipette to two sterile centrifuge tubes. These were centrifuged for five minutes at 1800 revolutions per minute, which treatment threw down only the coarser sediment. The supernatant liquid containing most of the bacteria was drawn off and put into other centrifuge tubes and again centrifuged thirty minutes at 2000 revolutions per minute. This produced a kind of differential sedimentation and is a process similar to that used by Strassburger[11] in his work on the occurrence of tubercle bacilli in the feces of men and also to that of MacNeal, Latzer, and Kerr[3] in their work on fecal bacteria of healthy men.

STAINING FECES SAMPLE FOR B. TUBERCULOSIS

The preparations for microscopical examinations were made by smearing the feces directly on slides. This was done either by picking up shreds of mucus from the surface of the fecal mass and spreading them on the slides, or by taking some of the mass itself and smearing it over the slide and rubbing it down to a thin layer with another slide. Besides the direct smears made in the two ways described, preparations of the sediment obtained by centrifugation were made and stained. All these preparations were stained in the manner described under general methods for staining *Bacillus tuberculosis* (page 324).

INOCULATION INTO TEST ANIMALS

Two guinea pigs were inoculated with each sample. The dose used for inoculation was in most instances one cubic centimeter of the original two percent suspension of feces. In a few instances 1cc. of a concentrated suspension of the mixed bacteria obtained by the centrifuge according to the method of Strasburger given above was injected. In all cases the injection was made into guinea pigs under the skin of the right thigh in doses of one cubic centimeter each. It is rather surprising that only a few guinea pigs died of acute infection when inoculated with the centrifuged bacteria. It is evident that centrifuging concentrates the fecal bacteria and among them the tubercle bacilli when present. An attempt was made to compare centrifuged sediment with

the original suspension by inoculating one guinea pig with the centrifuge sediment and another with one cubic centimenter of the two percent emulsion of the same sample. In no one of the twelve cases in which this was done have we found any tuberculosis. It is therefore impossible to estimate from these tests the relative merit of the two procedures. Only two of the twelve guinea pigs inoculated with centrifuged material died of acute infection and apparently one of these was not due to the bacteria inoculated but largely to some improper food. The results indicate that guinea pigs may stand a much larger dose of bacteria if they are free from other undissolved material.

DELICACY OF ANIMAL INOCULATION TESTS

Three series of experiments to ascertain the delicacy of guinea pig inoculation as a means of testing feces for tubercle bacilli have been performed. In these experiments definite amounts of a pure culture of bovine tubercle bacilli were added to suspensions of cow feces and then tested by injection into guinea pigs. The original of the cultures employed in the tests was obtained from Dr. Theobold Smith of Harvard Medical School, in October, 1908, and has since been propagated in our laboratory upon artificial media. The culture grows well on glycerin agar, glycerin potato, egg medium, and blood serum. It has been tested upon guinea pigs and shown to be virulent.

A heavy growth upon an inclined glycerin agar was used. The mass of bacterial substance was very carefully removed from the agar surface by means of a strong platinum wire, avoiding as far as possible the admixture of moisture or of agar. The bacterial substance was transferred to the inside of the neck of a weighed sterile 25 cc. measuring flask, and then accurately weighed upon an analytical balance. A little sterile 0.8 percent salt solution was then added and the mass thoroly rubbed up in the neck of the flask with a sterile glass rod. The glass rod and inner wall of the neck of the flask were carefully washed with salt solution as it was added to the contents of the flask from a Pasteur bulb pipette. By vigorous shaking and by further trituration of microscopic particles an apparently homogeneous suspension was obtained. It was then diluted to the mark on the flask (25 cc.). Microscopic examination showed most of the bacterial cells to be separate, but not all of them. Clumps of twenty or more cells and also some larger masses were still present. By allowing the suspension to settle a moment just before measuring out portions from it, these clumps were largely avoided in the material actually inoculated into the animals.

Experiment 1

For this experiment suspensions of a pure culture of bovine tubercle bacilli were desired in dilution of one to one hundred thousand and one to ten million. To make these suspensions 25 milligrams of the agar culture were weighed off and diluted accordingly.* The sample of feces was taken from heifer No. 73, which had given a negative tuberculin test. The results are recorded in Table 12. Every one of the guinea pigs in this experiment became tuberculous, even the controls. This was quite a disappointment, as far as the controls were concerned, for the feces were supposed to be free from tubercle bacilli. Immediately upon finding that the controls were tuberculous, another sample of feces was obtained from heifer 73, and four healthy guinea pigs inoculated with suspensions of the same. At the end of 83 days autopsy

TABLE 12.—THE QUANTITY OF TUBERCLE BACILLI IN FRESH COW MANURE NECESSARY TO PRODUCE TUBERCULOSIS WHEN INOCULATED INTO GUINEA PIGS

Material suspended, per cc.		Centrifugation	Guinea pig No.	Mode of death	Days elapsed	Autopsy	Microscopic exam.	Cultures
Feces mg.	Tubercle bacilli mg.							
0.	0.0100	0	201	Killed	42	Generalized tuberculosis	+	+
20.	0.0100	+	195	Killed	52	Generalized tuberculosis	+	+
20.	0.0100	+	196	Died	40	Generalized tuberculosis	+	+
20.	0.0100	0	193	Died	34	Generalized tuberculosis	+	+
20.	0.0100	0	194	Killed	47	Generalized tuberculosis	+	+
0.	0.0001	0	202	Killed	67	Generalized tuberculosis	+	+
20.	0.0001	+	199	Killed	42	Generalized tuberculosis	+	+
20.	0.0001	+	200	Killed	42	Generalized tuberculosis	+	+
20.	0.0001	0	197	Killed	42	Generalized tuberculosis	+	+
20.	0.0001	0	198	Killed	54	Generalized tuberculosis	+	+
20.	0.0000	0	191	Killed	42	Generalized tuberculosis	+	+
20.	0.0000	0	192	Killed	42	Generalized tuberculosis	+	+
Table 12a.—Supplementary test of Feces of Heifer 73								
20.	0.0000	0	273	Killed	83	Normal		
20.	0.0000	0	274	Killed	83	Normal		
20.	0.0000	0	275	Killed	83	Normal		
20.	0.0000	0	276	Killed	83	Normal		

showed them all to be healthy. It appears that by some mishap the control suspension contained some of the pure culture of tubercle bacilli. At the time there was available only one syringe and it is probable that the boiling which the syringe received between inoculations was insufficient to kill all the tubercle bacilli; and in this way some of the pure culture was carried over to the controls.

*Tubercle bacilli	Feces or salt solution.		Tubercle bacilli
0.025 g.	in 25 cc.	=Dil. A. 1 cc. contains	.001 g.
5 cc. Dil. A (.005 g.)	in 100 cc.	=Dil. B. 1 cc. contains	.000,05 g.
10 cc. Dil. B (.000, 5 g.)	in 50 cc.	=Dil. C. 1 cc. contains	.000,01 g.
0.5 cc. Dil. C (.000,005 g.)	in 50 cc.	=Dil. D. 1 cc. contains	.000,000,1 g.

Experiment 2

Two dilutions of bovine tubercle bacilli were used in this experiment, the more concentrated being the same as the most dilute in Experiment 1, i.e., one ten millionth gram of bovine tubercle bacilli per cubic centimeter; the highest dilution, one billionth gram per cubic centimeter. The fresh feces were obtained this time from heifer 74. She had just calved and was negative to the ophthalmic, cutaneous, and subcutaneous tuberculin tests. The sample was collected and suspensions made as described above. To make these suspensions 22 milligrams of germ substance from an agar culture were diluted as in Experiment 1.* In injecting these doses into the animals, three syringes were employed. These were sterilized in the autoclave at fifteen pounds pressure for fifteen minutes before using the first time. They were then sterilized by boiling for fully fifteen minutes before being used again The suspensions free from tubercle bacilli were injected first, then

TABLE 13.—THE QUANTITY OF TUBERCLE BACILLI IN FRESH COW MANURE NECESSARY TO PRODUCE TUBERCULOSIS WHEN INOCULATED INTO GUINEA PIGS. EXPERIMENT 2.

Material suspended, per cc.		Centrifugation	Guinea pig No.	Mode of death	Days elapsed	Autopsy	Microscopic exam.	Cultures
Feces mg.	Tubercle bacilli mg.							
20.	0.000 10	+	282	Killed	82	Generalized tuberculosis	+	+
20.	0.000 10	+	281	Died	73	Generalized tuberculosis	+	+
20.	0.000 10	0	280	Killed	82	Generalized tuberculosis	+	0
20.	0.000 10	0	279	Killed	82	Generalized tuberculosis	+	+
0.	0.000 10	0	287	Killed	82	Generalized tuberculosis	+	0
20.	0.000 01	+	286	Died	80	Generalized tuberculosis	+	0
20.	0.000 01	+	285	Killed	83	Generalized tuberculosis	+	0
20.	0.000 01	0	284	Killed	80	Normal	0	0
20.	0.000 01	0	283	Killed	81	Generalized tuberculosis	+	0
0.	0.000 01	0	289	Killed	82	Generalized tuberculosis	+	0
0.	0.000 01	0	288	Killed	82	Generalized tuberculosis	+	0
20.	0.000 00	0	278	Died	2	No tubercles	0	0
20.	0.000 00	0	277	Killed	82	Normal	0	0

*Tubercle bacilli — Feces or salt solution. — Tubercle bacilli

0.022 grams in 25 cc. =Dil. A, 1 cc. contains $\frac{.022}{25}$ g.

1 cc. of Dil. A ($\frac{.022}{25}$ g.) in 10 cc. =Dil. B, 1 cc. contains $\frac{.022}{250}$ g.

2.84 cc. of Dil. B ($\frac{.022 \times 2.84}{250}$ g.) in 50 cc. =Dil. C, 1 cc. contains $\frac{2.84 \times .022}{250 \times 50}$ g.

or 1 cc. contains .000,005 g.

1 cc. Dil. C (.000,005 g.) in 50 cc. =Dil. D, 1 cc. contains .000,000,1 g.

1 cc. Dil. C (.000,005 g.) in 100 cc. =Dil. E, 1 cc. contains .000,000,05 g.

1 cc. Dil. E (.000,000,05 g.) in 50 cc. =Dil. F, 1 cc. contains .000,000,001 g.

the suspensions containing the smaller amounts of these germs, and last those doses containing the larger amounts of tubercle bacilli. In this way the difficulty encountered in the preceding experiment was avoided. The results are recorded in Table 13. One of the controls died on the second day. The other survived and remained free from tubercles. Generalized tuberculosis was produced in the test guinea pigs by the highest dilution used, both by the pure culture samples and by the pure culture added to the feces. The centrifuged material produced on the whole a more severe infection.

Experiment 3

The feces suspension and emulsions of the pure culture of bovine tubercle bacilli were made in a similar manner to those in Experiment 2. Three dilutions of the tubercle bacilli were used in this experiment. The strongest concentration employed here was the same as the most dilute of Experiment 2, namely, one billionth gram pure culture of bovine tubercle bacilli per cubic centimeter. The second dilution contained one ten-billionth and the third dilution one-hundred-billionth gram of tubercle bacilli per cubic centimeter. To make these suspensions* 22.8 milligrams of the agar culture were diluted to the above amounts.

The results of these experiments are recorded in Table 14. In this experiment neither of the control guinea pigs became tuberculous. One, guinea pig 364, died of acute infection on the 11th day; the other was healthy at the time of autopsy 55 days after inoculation. At the time when this experiment was begun trouble existed with the stock guinea pigs dying on account of

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*Tubercle bacilli — Feces or salt solution — Tubercle bacilli

0.0228 gram in 25 cc. = Dil. A, 1 cc. contains $\frac{0.0228}{25}$ g.

1 cc. of Dil. A ($\frac{0.0228}{25}$ g.) in 100 cc. = Dil. B, 1 cc. contains $\frac{0.0228}{2500}$ g.

1 cc. of Dil. B ($\frac{0.0228}{2500}$ g.) in 100 cc. = Dil. C, 1 cc. contains $\frac{0.0228}{250000}$ g.

2.74 cc. of Dil. C ($\frac{2.74 \times 0.0228}{250000}$ g.) in 50 cc. = Dil. D, 1 cc. contains $\frac{2.74 \times 0.0228}{12{,}500{,}000}$ g. or 0.000,000,005 gram

10 cc. of Dil. D (0.000,000,05 g.) in 50 cc. = Dil. E, 1 cc. contains 0.000,000,001 g.

1 cc. of Dil. D (0.000,000,005 g.) in 50 cc. = Dil. F, 1 cc. contains 0.000,000,000,1 g.

$\frac{1}{10}$ cc. of Dil. D (0.000,000,000,5 g.) in 50 cc. = Dil. G, 1 cc. contains 0.000,000,000,01 g.

TABLE 14.—THE QUANTITY OF TUBERCLE BACILLI IN FRESH COW MANURE NECESSARY TO PRODUCE TUBERCULOSIS WHEN INOCULATED INTO GUINEA PIGS. EXPERIMENT 3.

Material suspended, per cc.		Centrifugation	Guinea pig No.	Mode of death	Days elapsed	Autopsy	Microscopic exam.	Cultures
Feces mg.	Tubercle bacilli mg.							
20.	0.000 001	+	369	Died	10	No tubercles	.....	.....
20.	0.000 001	+	368	Died	1	Died within a few hours	.....	.....
20.	0.000 001	0	367	Died	10	No tubercles	0	.....
20.	0.000 001	0	366	Died	13	No tubercles	0	.....
0.	0.000 001	0	378	Killed	54	Generalized tuberculosis	+	0
20.	0.000 000 10	+	373	Died	1	No tubercles	0	.....
20.	0.000 000 10	+	372	Killed	53	Generalized tuberculosis	+	0
20.	0.000 000 10	0	371	Died	6	No tubercles	.....	.....
20.	0.000 000 10	0	370	Killed	53	Normal	.....	.....
0.	0.000 000 10	0	379	Died	6	No tubercles	.. .	.....
20.	0.000 000 01	+	377	Died	1	No tubercles	0	.. ...
20.	0.000 000 01	+	376	Killed	53	Generalized tuberculosis	+	.....
20.	0.000 000 01	0	375	Died	5	No tubercles	0	.....
20.	0.000 000 01	0	374	Killed	53	Slight tuberculosis	+	.....
0.	0.000 000 01	0	381	Died	3	No tubercles	.....	.....
0.	0.000 000 01	0	380	Died	0	Died when inoculated	.....	.....
20.	0.000 000 00	0	365	Killed	55	Normal	... ..	.....
20.	0.000 000 00	0	364	Died	11	No tubercles	0	0

improper care. One of the animals selected for this experiment, guinea pig 380, died after it was brought into the room just before time for inoculation, and within 8 or 10 days one-half the guinea pigs in the experiment were dead. So this experiment was almost a failure. However, we find even the greatest dilution causing generalized tuberculosis (No. 376).

A decided difference was observed between centrifuged and uncentrifuged samples. Note in this connection guinea pig 370, inoculated with a sample that was not centrifuged, having a dilution of one-ten billionth gram of pure culture of bovine tubercle bacilli. This guinea pig remained healthy, while guinea pig 372, inoculated with the sample of the same dilution but centrifuged, contracted generalized tuberculosis. Also note that guinea pig 374, inoculated from the sample whose dilution was one-one-hundred-billionth gram per cubic centimeter, which was not centrifuged, had only slight tuberculosis; while guinea pig 376, inoculated from the sediment of the same sample after centrifugation had generalized tuberculosis.

Conclusions

1. A pure culture of bovine tubercle bacilli in quantities as small as one one-hundred-billionth gram suspended in sterile salt solution or in one percent feces emulsion produced tuberculosis in guinea pigs.

2. When the dilutions of pure culture of bovine tubercle bacilli were as high as one-hundred-billionth gram per cubic centimeter of feces suspension the subcutaneous inoculation of the sediment of 20 cc. of the centrifuged suspension produced a more extensive generalized tuberculosis than did a similar inoculation of one cubic centimeter of the same suspension not centrifuged. In dilutions containing a greater amount of germ substance this distinction was not so clear, as very extensive generalized tuberculosis usually resulted from the injection of even the one cubic centimeter of the uncentrifuged material.

3. No difference was noted in the effect upon guinea pigs whether the pure culture was inoculated in one cubic centimeter of sterile salt solution or in one cubic centimeter of one percent fresh feces suspension. Possibly higher dilutions may determine this point.

MICROSCOPIC EXAMINATION OF FECES FOR TUBERCLE BACILLI

METHOD OF PROCEDURE

Before obtaining the first shipment of guinea pigs which were to be used in this work, a considerable number of miscroscopic tests were made. The samples were collected as previously described for the first series of tests. Two samples were taken from each cow, a mass sample of feces picked up from the floor after the cow had voided, and a sample scraped from the wall of the rectum by means of a sterilized bone spoon. From these two samples, three microscopic preparations were made. Shreds of mucus were first removed from the surface of the mass of feces and spread out on glass slides, fixed and stained. Then the mass was thoroly mixed and a smear of this mixed material made on a slide, fixed and stained. A third preparation was made by smearing the mucus fluid obtained from the rectum on a slide. All three of these smears were stained with carbol-fuchsin and methylene blue as described under General Methods (page 324). Each slide was examined 15 minutes and the number of acid-fast organisms recorded as shown in the Tables 15 and 16. If no acid-fast organisms were found in any of the slides made from the sample tested, it was recorded as a negative result. Usually only a few acid-fast organisms were found on one slide in the 15 minutes. Only five times from the 25 slides examined were as many as 25 acid-fast organisms found on a slide. At no time were shreds of tissue containing abundant acid-fast organisms found; the bacilli were generally separate. An acid-fast streptothrix was found in one sample of feces from cow No. 12. Often oval spore-like acid-fast bodies were found. These in no way re-

sembled *Bacillus tuberculosis* and were never included as positive findings in the results tabulated. The only acid-fast organisms so included were short, thin rods which were often slightly curved, but sometimes quite straight. In appearance, they resembled individual rods of the bovine tubercle bacillus, a slide of which was constantly kept at hand for comparison.

RESULTS

The feces of both reacting and non-reacting cows were examined microscopically for tubercle bacilli. Table 15 gives the results from the reacting cows. Altogether 119

TABLE 15.—MICROSCOPIC EXAMINATION FOR TUBERCLE BACILLI IN THE FECES OF REACTING COWS

Cow No.	Sample No.	Date of examination	A. Mass of feces			B. Scraping from the rectum	Summary of test	Summary of test on cow	
			1. External mucus	2. Mixed material	3. Centrifuged sediment			Pos.	Neg.
10	1	6-29-08	2	0	... ...	only one	+		
	2	8-20-08	.....	.....	0	.....	0	1	1
12	1	6-18-08	0	0	.....	0 *	0		
	2	7- 1-08	many	.....	.....	0	+		
	3	4-28-08	.....	.....	0	.....	0	1	2
13	1	6-18-08	24	some	.....	several	+		
	2	6-22-08	several	6	... .	several	+		
	3	7- 1-08	....	.....	.....	4	+		
	4	7-11-08	.....	.....	.....	several	+		
	5	8- 6-08	.....	.....	0	.....	0		
	6	8-12-08	.....	.....	0	.....	0		
	7	8-19-08	... ...	.....	0	.....	0		
	8	9- 2-08	....	.....	several	.....	+	5	3
15	1	6-19-08	0	2	.....	.....	+		
	2	8-28-08	.....	.....	0	.....	0	1	1
16	1	6-24-08	0	2	... ...	3	+		
	2	8-13-08	.....	... ...	0	.....	0	1	1
19	1	6-28-08	0	1	.....	few	+		
	2	8-18-08	....	... ...	0	.....	0	1	1
28	1	6-18-08	25	some	.....	.....	+		
	2	6-22-08	several	several	.....	12	+		
	3	7- 1-08	.....	.....	.....	12	+		
	4	7-11-08	2	.....	.....	.....	+		
	5	8- 6-08	.....	.....	2	.....	+		
	6	8-12-03	. ....	....	1	.....	+		
	7	8-18-08	.....	.....	0	.....	0		
	8	9- 2-08	.....	....	many	.....	+	7	1
29	1	6-29-08	1	5	.....	3	+		
	2	7- 1-08	0	.....	.....	0	0		
	3	9- 2-08	.....	.....	0	.....	0		
	4	10-30-09	.....	.....	0	....	0		
	5	11-13-09	... ...	.....	0	.....	0	1	4
33	1	6-25-08	0	3	.....	2	+		
	2	8-14-08	.....	.....	0	....	0	1	1
35	1	6-19-08	0	.....	.....	0	0		
	2	8- 6-08	.....	... .	0	.....	0		
	3	9- 3-08	.....	.. ....	many	....	+		
	4	11-13-09	.... ..	. ....	0	....	0	1	3
36	1	6-19-08	several	several	.... ..	several	+		
	2	6-24-08	few	1	.....	. 2	+		
	3	8-13-08	.....	.....	0	....	0	2	1

*Acid-fast streptothrix.

TABLE 15.—*Continued*

Cow No.	Sample No.	Date of examination	A. Mass of feces: 1. External mucus	A. Mass of feces: 2. Mixed material	A. Mass of feces: 3. Centrifuged sediment	B. Scraping from the rectum	Summary of test	Summary of test on cow: Pos.	Summary of test on cow: Neg.
37	1	6-19-08	0	0	.....	0	0		
	2	8-28-08	.. ....	.....	0	.. ..	0	0	2
39	1	6-23-08	few	2	.. ....	3	+		
	2	8-25-08	0	.. ....	.....	.....	0	1	1
41	1	6-29-08	2	4	.....	few	+		
	2	9- 3-08	.....	.....	0	.....	0	1	1
42	1	6-19-08	0	.. ....	.... .	0	0		
	2	8- 6-08	.....	.....	0	.....	0		
	3	9- 3-08	.....	.. ....	0	.....	0		
	4	10-30-09	.....	... .	0	.....	0	0	4
47	1	6-29-08	.....	6	.... ..	2	+		
	2	9- 3-08	... .	.....	0	... .	0	1	1
49	1	6-24-08	12	0	... .	0	+		
	2	8-26-08	.....	.....	0	.....	0	1	1
51	1	6-26-08	0	0	.....	0	0		
	2	8-18-08	.....	.....	0	.....	0	0	2
52	1	6-22-08	4	several	.....	several	+		
	2	7- 1-08	8	.....	.....	.....	+		
	3	8-21-08	.. ....	.....	1	0	+	3	0
53	1	6-25-08	several	several	.....	several	+		
	2	8-14-08	.....	.....	0	....	0		
	3	8-18-08	.....	.....	0	.....	0		
	4	9- 2-08	.. ....	... .	many	....	+	2	2
54	1	8-25-08	.....	....	0	.....	0	0	1
55	1	6-29-08	3	many	.. ...	2	+		
	2	8-27-08	.....	.... ..	2	.....	+		
	3	11-13-09	.....	.....	1	....	+	3	0
56	1	6-25-08	6	several	....	3	+		
	2	8-14-08	....	.....	0	....	0		
	3	8-30-09	.....	.....	0	....	0		
	4	11-13-09	. .....	.....	1	.....	+	2	2
57	1	6-25-08	several	several	.....	several	+		
	2	8-14-08	.....	.....	0	.....	0	1	1
58	1	6-26-08	few	few	.....	0	+		
	2	8-18-08	.....	.....	0	.. ..	0	1	1
59	1	8-18-08	.. ....	.....	0	....	0	0	1
60	1	6-24-08	26	24	.....	several	+		
	2	8-13-08	... .	.....	0	.....	0	1	1
61	1	8-11-08	.....	.. ....	0	.....	0		
	2	8-27-08	.....	.....	0	.....	0	0	2
62	1	6-29-08	3	25	.....	.....	+		
	2	7- 1-08	0	....	0	0	0	1	1
63	1	9- 2-08	.....	.....	many	.....	+	1	0
64	1	8-20-08	.....	.....	0	....	0		
	2	8-30-09	.....	.....	0	.....	0	0	2
66	1	8-20-08	.....	... ...	0	.....	0	0	1
67	1	8-21-08	.....	.....	0	.....	0	0	1
70	1	8-11-08	.....	.. ...	0	.....	0		
	2	9- 2-08	.....	.. ....	many	.....	+	1	1
72	1	8-30-09	.....	.....	1	.....	+	1	0
76	1	8-25-08	.....	.....	0	.....	0	0	1
80	1	9- 2-08	.....	.....	2	.....	+	1	0

TABLE 15.—*Continued*

Cow No.	Sample No.	Date of examination	A. Mass of feces			B. Scraping from the rectum	Summary of test	Summary of test on cow	
			1. External mucus	2. Mixed material	3. Centrifuged sediment			Pos.	Neg.
83	1	6-26-08	3	2	.....	2	+		
	2	6-29-08	1	13	.....	few	+		
	3	8-20-08	.....	.....	0	.....	0	2	1
86	1	6-23-08	several	several	.....	2	+	1	0
87	1	6-19-08	0	.....	.....	0	0		
	2	6-23-08	few	few	.....	2	+		
	3	8-21-08	.....	.....	4	.....	+	2	1
97	1	6-22-08	12	+	.....	2	+		
	2	8-21-08	.....	.....	0	.....	0	1	1
98	1	6-23-08	0	1	.....	few	+	1	0
109	1	9-30-09	.....	.....	1	.....	+	1	0
S.H.	1	9-30-09	.....	.....	0	.....	0		
	2	11-13-09	.....	.....	0	.....	0	0	2
L.H.	1	9-30-09	.....	.....	5	.....	+	1	0
L.B.	1	11-13-09	.....	.....	0	.....	0		
	2	11-28-09	.....	.....	0	.....	0	0	2
W.	1	11-13-09	.....	.....	0	.....	0		
	2	11-28-09	.....	.....	0	.....	0	0	2
S6	1	11-13-09	.....	.....	0	.....	0		
	2	11-26-09	.....	.....	0	.....	0	0	2
F.	1	11-15-09	.....	.....	0	.....	0		
	2	11-26-09	.....	.....	0	.....	0	0	2
O.S.	1	11-15-09	.....	.....	0	.....	0	0	1
B.	1	11-15-09	.....	.....	0	.....	0	0	1
O.C.	1	11-15-09	.....	.....	0	.....	0	0	1
O.	1	11-15-09	.....	.....	0	.....	0	0	1
Total, 53 cows, 119 samples								53	66

samples were tested from 53 cows. Acid-fast organisms were found 53 times; 66 times there were no such organisms found. Of the 53 reacting cows tested, acid-fast bacilli were at some time found in the feces of all but 19. Of these 19 in which such bacteria were not found at all, 8 were tested only once, 9 twice, and 1 four times. Table 16 gives the results of examining the feces from 18 non-reacting cows. Forty samples were tested of which 23 were found to have acid-fast organisms and in 17 none were found. From 3 of the 18 cows no samples of feces examined contained acid-fast organisms. From two of these, two samples were tested, from the other, only one sample was used. Thus, 28 per cent of the 53 reacting cows gave entirely negative results, while only 16⅔ per cent of the non-reacting herd gave samples without acid-fast organisms. It would seem from this result that no significance can be attached to the microscopic finding of a few acid-fast bacteria in the feces of cattle.

In this connection it is interesting to note the opinions of the following authors: Schroeder and Cotton[9] think that the micro-

TABLE 16.—MICROSCOPIC EXAMINATION FOR TUBERCLE BACILLI IN THE FECES OF NON-REACTING COWS

Cow No.	Sample No.	Date of examination	A. Mass of feces: External mucus	A. Mass of feces: Mixed material	A. Mass of feces: Centrifuged sediment	B. scraping from the rectum	Result of test	Summary of tests on cow: Pos.	Summary of tests on cow: Neg.
A	1	6-29-'08	0	several	.....	+	+		
	2	8-27-'08	.....	.....	14	.....	+		
	3	8-28-'08	.....	.....	6	.....	+	3	0
B	1	6-29-'08	0	0	.....	several	+		
	2	7- 1-'08	0	.....	.....	0	0		
	3	8-12-'08	.....	.....	0	.....	0		
	4	8-27-'08	.....	.....	0	.....	0	1	3
C	1	6-29-'08	3	0	.....	4	+		
	2	7- 1-'08	0	.....	.....	0	0		
	3	8-27-'08	.....	.....	0	.....	0	1	2
D	1	8- 7-'08	.....	.....	0	.....	0		
	2	9- 1-'08	.....	.....	2	.....	+	1	1
E	1	8-12-'08	.....	.....	0	.....	0		
	2	9- 1-'08	.....	.....	0	.....	0	0	2
F	1	8- 7-'08	.....	.....	1	.....	+		
	2	9- 1-'08	.....	.....	0	.....	0	1	1
G	1	8- 7-'08	.....	.....	0	.....	0		
	2	9- 1-'08	.....	.....	4	.....	+	1	1
H	1	8- 6-'08	.....	.....	3	.....	+		
	2	9- 1-'08	.....	.....	2	.....	+	2	0
I	1	6-22-'08	24	several	.....	several	+		
	2	8-25-'08	.....	.....	0	.....	0	1	1
24	1	6-29-'08	5	few	.....	2	+		
	2	7- 1-'08	0	.....	.....	0	0		
	3	7-28-'08	.....	.....	0	.....	0	1	2
26	1	6-29-'08	3	1	.....	0	+		
	2	7- 1-'08	0	.....	.....	0	0	2	1
	3	7-28-'08	.....	.....	3	.....	+		
65	1	9- 2-'08	.....	.....	0	.....	0	0	1
68	1	9- 2-'08	.....	.....	many	.....	+	1	0
69	1	9- 2-'08	.....	.....	0	.....	0		
	2	8-11-'08	.....	.....	0	.....	0	0	2
85	1	7- 1-'08	4	.....	.....	.....	+		
	2	8-27-'08	.....	.....	1	.....	+	2	0
R	1	7-16-'08	.....	3	.....	3	+		
	2	7-28-'08	4	4	.....	6	+	2	0
H	1	7-16-'08	0	2	.....	2	+		
	2	7-28-'08	1	1	.....	0	+	2	0
J	1	7-16-'08	0	2	.....	2	+		
	2	7-28-'08	0	1	.....	4	+	2	0
Total 18 cows, 40 samples.								23	17

scope is adequate in determining the presence of tubercle bacilli in feces of cattle. Reynolds and Beebe[5] were not able to find the tubercle bacilli in the feces of cow No. 2½ with which they did produce tuberculosis in guinea pigs by subcutaneous inoculations. They think the microscopic method is not a reliable test for tubercle bacilli in cattle manure. Peters and Emerson[4] do not think that a

microscopical test is a sufficient means of testing for tubercle bacilli in the feces of a cow. Out of twenty-two cows in the feces of which they had found acid-fast organisms by microscopic test, guinea pig inoculations only showed three to be passing virulent tubercle bacilli. Bang[1] says the microscopic test is of no value except for rapid work when the test is positive and the tubercle bacilli are found in abundance; as in the case of tuberculosis of the udder, where a stained preparation of a sample of milk may reveal hundreds of tubercle bacilli.

ANIMAL INOCULATION TESTS FOR TUBERCLE BACILLI IN THE FECES OF REACTING CATTLE

INOCULATION TESTS

The method of collecting the samples and preparing the feces suspension for inoculation has been given (see page 367.) The amount inoculated was one cubic centimeter of a one percent suspension of the feces. Only a few times was the suspension centrifuged and the sediment used for inoculation. Two guinea pigs were used for each sample, with few exceptions, when three were used. There have been made two complete series of tests including all the reacting dairy cattle present in the herd at the times these tests were made: The first series was made between the 28th of December, 1908, and the 4th of January, 1909. At this time samples from thirty-eight cows were tested and two were found to be passing virulent tubercle bacilli, cow No. 1004 and cow No. 83. The two guinea pigs inoculated from each were found to have generalized tuberculosis. The diagnosis of tuberculosis in the guinea pigs was confirmed by microscopical examination, by cultures, and by inoculations of the tuberculous tissues of each of the four guinea pigs into two other guinea pigs. All of these tests were characteristic.

The second series of tests was made between August 6 and August 9, 1909. Three weeks later it was noted that guinea pigs inoculated from samples of cows 72, 64, and 56 had enlarged superior inguinal lymphatic glands and for this reason another sample was collected from each of these three cows and inoculated into guinea pigs on August 31. Curiously enough the swelling of the lymph glands in the guinea pigs inoculated August 6-9 subsided, and at autopsy some weeks later, no evidence of tuberculosis was found in them. However, of the guinea pigs inoculated August 31 as a supplementary test, two, Nos. 527 and 528, inoculated with feces of cow 56 became tuberculous. No other guinea pigs inoculated in this series of tests showed any evidence of tuberculosis at autopsy. Cow 56 was retested August 16, 1910, by inoculating two guinea pigs, 778 and 779, subcutaneously. One of these guinea pigs, 779, was wholly negative, the other, 778, became tuberculous and the

TABLE 17.—ANIMAL INOCULATION TESTS FOR TUBERCLE BACILLI IN THE FECES OF REACTING COWS

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic	Cultures	Reinoculation
10	1	252	2-4-'09	Killed	52	Normal			
		253	2-4-'09	Killed	52	Normal			
	2	512	8-9-'09	Killed	40	Normal			
		513	8-9-'09	Killed	40	Normal			
	3	640*	9-22-'09	Killed	14	Normal			
		641*	9-22-'09	Killed	14	Normal			
12	1	254	2-4-'09	Killed	52	Normal			
		255	2-4-'09	Killed	52	Normal			
13	1	78	8-19-'08	Killed	61	Normal			
		79	8-19-'08	Killed	61	Normal			
		80	8-19-'08	Killed	61	Normal			
	2	116	9-2-'08	Died	2	Acute infection			
		117	9-2-'08	Killed	62	Normal			
		118	9-2-'08	Killed	62	Normal			
	3	173	12-28-'08	Killed	54	Normal			
		174	12-28-'08	Killed	55	Normal			
15	1	187	12-29-'08	Killed	54	Normal			
		188	12-29-'08	Killed	54	Normal			
16	1	203	12-31-'08	Killed	53	Normal			
		204	12-31-'08	Killed	53	Normal			
	2	492	8-7-'09	Killed	39	Normal			
		493	8-7-'09	Killed	39	Normal			
19	1	232	2-3-'09	Killed	51	Normal			
		233	2-3-'09	Killed	51	Normal			
28	1	81	8-19-'08	Died	2	Acute infection			
		82	8-19-'08	Killed	59	Normal			
		83	8-19-'08	Killed	59	Normal			
	2	119	9-2-'08	Killed	62	Normal			
		120	9-2-'08	Killed	62	Normal			
		121	9-2-'08	Killed	62	Normal			
29	1	663	10-30-'09	Died	1	Acute infection			
		664	10-30-'09	Died	1	Acute infection			
	2	680	11-13-'09	Killed	51	Normal			
		681	11-13-'09	Died	6	Acute infection			
33	1	205	12-31-'08	Killed	53	Normal			
		206	12-31-'08	Killed	53	Normal			
	2	498	8-7-'09	Killed	41	Normal			
		499	8-7-'09	Killed	41	Normal			
35	1	682	11-13-'09	Died	12	Acute infection	organisms not acid fast	organisms not acid fast	
		681	11-13-'09	Killed	51	Normal			
36	1	207	12-31-'08	Killed	53	Abscess in lung (no tubercles)	organisms not acid fast	organisms not acid fast	
		208	12-31-'08	Killed	53	Normal			
	2	502	8-7-'09	Killed	42	Normal			
		503	8-7-'09	Killed	42	Normal			
37	1	177	12-29-'08	Killed	53	Normal			
		178	12-29-'08	Killed	53	Normal			
	2	642*	9-22-'09	Killed	14	Normal			
		643*	9-22-'09	Killed	14	Normal			

*Killed by a dog.

TABLE 17—*Continued*

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic	Cultures	Reinoculation
39	1	181	12-29-'08	Killed	54	Normal			
		182	12-29-'08	Killed	54	Normal			
41	1	209	12-31-'08	Killed	53	Normal			
		210	12-31-'08	Killed	53	Normal			
	2	506	8-9-'09	Killed	40	Normal			
		507	8-9-'09	Killed	40	Normal			
42	1	665	10-30-'09	Died	1	Acute infection			
		666	10-30-'09	Killed	64	Normal			
47	1	37	9-15-'09	Died	50	Unknown cause (no tubercles)	Organisms not acid fast		
		138	9-15-'08	Killed	107	Normal			
		139	9-15-'08	Died	57	Unknown cause (no tubercles)	Organisms not acid fast		
49	1	211	12-31-'08	Killed	53	Normal			
		212	12-31-'08	Killed	53	Normal			
	2	496	8-7-'09	Killed	49	Normal			
		497	8-7-'09	Killed	49	Normal			
51	1	508	8-9-'09	Killed	40	Normal			
		509	8-9-'09	Killed	40	Normal			
52	1	256	2-4-'09	Killed	52	Normal			
		257	2-4-'09	Killed	52	Normal			
	2	486	8-6-'09	Killed	39	Normal			
		487	8-6-'09	Killed	39	Normal			
	3	620	9-18-'09	Killed	60	Normal			
53	1	84	8-19-'08	Killed	59	Normal			
		85	8-19-'08	Killed	59	Normal			
	2	86	8-19-'08	Died	3	Acute infection			
		122	9-2-'08	Killed	63	Normal			
	3	123	9-2-'08	Killed	63	Normal			
		124	9-2-'08	Killed	63	Normal			
54	1	185	12-29-'08	Killed	54	Normal			
		186	12-29-'08	Killed	54	Normal			
	2	480	8-6-'09	Killed	39	Normal			
		481	8-6-'09	Killed	39	Normal			
	3	621	8-18-'09	Killed	60	Normal			
		622	8-18-'09	Killed	60	Normal			
		623	8-18-'09	Killed	60	Normal			
55	1	684	11-13-'09	Died	5	Acute infection			
		685	11-13-'09	Killed	51	Normal			
56	1	213	12-31-'08	Killed	53	Normal			
			12-31-'08	Killed	53	Normal			
	2	520	8-9-'09	Killed	44	Normal			
		521	8-9-'09	Killed	44	Normal			
	3	527	8-31-'09	Killed	28	Tuberculosis (superior inguinal)	+	+	++
		528	8-31-'09	Killed	28	Tuberculosis (superior inguinal)	+	---	---
	4	652	10-4-'09	Killed	53	Normal			
		653	10-4-'09	Killed	53	Normal			

TABLE 17.—*Continued*

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic	Cultures	Reinoculation
56	5	678	11-13-'09	Died	9	Acute infection	Organisms not acid fast	Organisms not acid fast	
		679	11-13-'09	Killed	51	Normal			
	6	778	8-16-'10	Killed	80	Slight tuberculosis	+	+	++
		779	8-16-'10	Killed	143	Normal	—	—	—
57	1	258	2-4-'09	Killed	52	Normal			
		259	2-4-'09	Killed	52	Normal			
	2	484	8-6-'09	Killed	39	Normal			
		485	8-6-'09	Killed	39	Normal			
	3	644	9-22-'09	Killed	57	Normal			
		645	9-22-'09	Killed	57	Normal			
58	1	260	2-4-'09	Killed	52	Normal			
		261	2-4-'09	Killed	52	Normal			
	2	518	8-9-'09	Killed	44	Normal			
		519	8-9-'09	Killed	44	Normal			
59	1	262	2-4-'09	Killed	52	Normal			
		263	2-4-'09	Killed	52	Normal			
	2	524	8-9-'09	Killed	44	Normal			
		525	8-9-'09	Killed	44	Normal			
60	1	234	2-3-'09	Killed	51	Normal			
		235	2-3-'09	Killed	51	Normal			
	2	500	8-7-'09	Killed	41	Normal			
		501	8-7-'09	Killed	41	Normal			
63	1	514	8-9-'09	Killed	44	Normal			
		515	8-9-'09	Killed	44	Normal			
64	1	215	12-31-'08	Killed	53	Normal			
		216	12-31-'08	Killed	53	Normal			
	2	478	8-6-'09	Killed	39	Normal			
		479	8-6-'09	Killed	39	Normal			
	3	531	8-31-'09	Killed	28	Normal			
		532	8-31-'09	Killed	28	Normal			
66	1	217	12-31-'08	Killed	53	Normal			
		218	12-31-'09	Killed	53	Normal			
	2	488	8-6-'09	Killed	39	Normal			
		489	8-6-'09	Killed	39	Ulcer at point of inoculation (no tubercles)	Organisms not acid fast	Organisms not acid fast	
67	1	264	2-4-'09	Killed	52	Normal			
		265	2-4-'09	Killed	52	Normal			
	2	490	8-6-'09	Killed	38	Normal			
		491	8-6-'09	Killed	38	Normal			
70	1	516	8-9-'09	Killed	44	Normal			
		517	8-9-'09	Killed	44	Normal			
71	1	189	12-29-'08	Killed	54	Normal			
			12-29-'08	Killed	54	Normal			
	2	482	8-6-'09	Killed	39	Normal			
		483	8-6-'09	Killed	39	Normal			
72	1	183	12-29 '08	Killed	54	Normal			
		184	12-29-'08	Killed	54	Normal			
	2	476	8-6-'09	Killed	39	Normal			
		477	8-6-'09	Died	10	Acute infection (no tubercles)	Organisms not acid fast		

TABLE 17.—*Continued*

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic	Cultures	Reinoculation
72	3	529	8-31-'09	Killed	28	Normal			
		530	8-31-'09	Died	2	Acute infection			
	4	547	9-4-'09	Killed	39	Normal			
		548	9-4-'09	Killed	39	Normal			
73	1	522	8-9-'09	Killed	44	Normal.			
		523	8-9-'09	Killed	44	Normal			
76	1	219	12-31-'08	Killed	53	Normal			
		220	12-31-'08	Killed	53	Normal			
77	1	169	12-28-'08	Killed	54	Normal			
		170	12-28-'08	Killed	54	Normal			
78	1	624	9-18-'09	Killed	60	Normal			
		625	9-18-'09	Killed	60	Normal			
79	1	171	12-28-'08	Killed	54	Normal			
		172	12-28-'08	Killed	54	Normal			
80	1	266	2-4-'09	Killed	52	Normal			
		267	2-4-'09	Killed	52	Normal			
83	1	268	2-4-'09	Killed	52	Gen. tuber.	+	+	++
		269	2-4-'09	Killed	52	Gen. tuber.	+	+	−
89	1	510	8-9-'09	Killed	40	Normal			
		511	8-9-'09	Killed	40	Normal			
95	1	179	12-29-'08	Killed	54	Normal	No acid-fast organisms	No acid-fast organisms	
		180	12-29-'08	Killed	24	Tumor on liver (No tubercles.)			
104	1	236	2-3-'09	Killed	51	Normal			
		237	2-3-'09	Killed	51	Normal			
109	1	494	8-7-'09	Killed	39	Normal			
		495	8-7-'09	Killed	39	Normal			
	2	667	10-30-'09	Killed	64	Normal			
		668	10-30-'09	Died	1	Acute infection			
1002	1	221	12-31-'08	Killed	53	Normal			
		222	12-31-'08	Killed	53	Normal			
1004	1	223	12-31-'08	Killed	53	Gen. tuber.	+	+	+
		224	12-31-'08	Killed	53	Gen. tuber.	+	+	+
1007	1	238	2-3-'09	Killed	51	Normal			
		239	2-3-'09	Killed	51	Normal			
1008	1	240	2-3-'09	Killed	51	Normal			
		241	2-3-'09	Killed	51	Normal			
1014	1	242	2-3-'09	Killed	50	Normal			
		243	2-3-'09	Killed	50	Normal			
1017	1	244	2-3-'09	Killed	51	Normal			
		245	2-3-'09	Killed	51	Normal			
G.H.	1	246	2-3-'09	Killed	51	Normal			
		247	2-3-'09	Killed	51	Normal			
S. H.	1	671	10-30-'09	Died	1	Acute infection			
		672	10-30-'09	Died	2	Acute infection			
	2	676	11-13-'09	Died	9	Acute infection			
		677	11-13-'09	Killed	51	Normal			

TABLE 17—*Continued*

Cow No.	Sample No.	Guinea pig No.	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Microscopic	Cultures	Reinoculation
L.H.	1	669	10-30-'09	Died	1	Acute infection			
		670	10-30-'09	Killed	64	Normal			
L. B.	1	686	11-15-'09	Died	13	Acute infection			
		687	11-15-'09	Died	13	Acute infection			
	2	710	12-1-'09	Killed	33	Normal			
		711	12-1-'09	Killed	33	Normal			
W.E	1	688	11-15-'09	Died	13	Acute infection			
		689	11-15-'09	Died	13	Acute infection			
	2	708	12-1-'09	Killed	33	Normal			
		709	12-1-'09	Killed	33	Normal			
S. 6.	1	690	11-15-'09	Died	3	Acute infection			
		691	11-15-'09	Died	3	Acute infection			
	2	702	11-25-'09	Killed	39	Normal			
		703	11-25-'09	Killed	39	Normal			
F. S.	1	692	11-16-'09	Killed	49	Normal			
		693	11-15-'09	Killed	49	Normal			
O.S.	1	694	11-15-'09	Died	6	Acute infection			
		695	11-15-'09	Killed	49	Normal			
O. C.	1	698	11-11-'09	Killed	49	Normal			
		699	11-15-'09	Died	4	Acute infection			
B.	1	696	11-15-'09	Killed	49	Normal			
		697	11-15-'09	Killed	49	Normal			
C.E.	1	700	11-15-'09	Died	3	Acute infection			
		701	11-15-'09	Killed	49	Normal			

lesions gave staining, culture and reinoculation tests characteristic for tubercle bacilli. There has been at irregular times a number of other tests for tubercle bacilli in the feces of these same and of other cattle; included in these were the tests of eight beef cattle. Tubercle bacilli were not found in any sample in these tests. The results of all the tests are shown in Table 17. Altogether, ninety-seven samples from sixty-two cattle have been tested. (For Autopsy records of these cattle see Table 19.) In the feces of three of these, or (practically) five percent, virulent tubercle bacilli were present at the time, as shown by guinea pig inoculation.

COMPARISON WITH THE MICROSCOPIC FINDINGS

There is given in Table 18 a summary and comparison of the microscopic examination and animal inoculation test for tubercle bacilli in the feces of reacting cows. From this summary there seems to be no relation between the presence of acid-fast organisms found on a microscopic slide and the presence of viru-

lent tubercle bacilli as proved by guinea pig inoculation. Our results are very similar to those of Reynolds and Beebe[5] and of Peters and Emerson[4]. They are unlike the results of Schroeder and Cotton[9] and of the Royal Commission on Tuberculosis of England.[6] The latter two found tubercle bacilli in the feces in a much higher percentage of tests. The percentage of positive cases that Schroeder and Cotton found was based upon microscopic examination. However they demonstrated by guinea pig inoculation that cattle do pass virulent tubercle bacilli in their feces. The six cattle tested by the Royal Commission and possibly also those used by Schroeder and Cotton are not comparable in respect to the extent of infection to the large herds used in the experiments of Reynolds and Beebe, Peters and Emerson, and ourselves. We take it that the latter three herds are much more representative of the tuberculous cattle thruout this country. More work must be done before final conclusions are reached.

Whether the percentage of cattle passing virulent tubercle bacilli in the feces is relatively large or relatively small, would appear to depend upon the general condition of the herd and the stage of the disease in its individual members. Agricultural Experiment Station herds are probably above the average in the general condition of the animals and in freedom from emaciated and visibly diseased individuals. On the other hand, certain selected herds

TABLE 18.—SUMMARY AND COMPARISON OF THE MICROSCOPIC EXAMINATION AND ANIMAL INOCULATION TESTS FOR TUBERCLE BACILLI IN THE FECES OF REACTING COWS

Cow No.	Results with microscope		Results with guinea pig		Cow No.	Results with microscope		Results with guinea pig	
	Positive	Negative	Positive	Negative		Positive	Negative	Positive	Negative
10	1	1	0	3	54	0	1	0	3
12	1	2	0	1	55	2	1	0	1
13	5	3	0	3	56	2	2	2	4
15	1	0	0	1	57	1	1	0	3
16	1	0	0	2	58	1	1	0	2
19	1	0	0	1	59	0	1	0	2
28	7	0	0	3	60	1	1	0	2
29	1	4	0	2	61	0	2	....	....
33	1	1	0	1	62	1	2	....	....
35	1	3	0	1	63	1	0	0	1
36	2	1	0	2	64	0	2	0	3
37	0	2	0	2	66	0	1	0	2
39	1	1	0	1	67	0	1	0	2
41	1	1	0	2	70	1	1	0	1
42	0	4	0	1	71	....	....	0	2
47	1	1	0	1	72	1	0	0	4
49	1	1	0	2	73	....	....	0	1
51	0	2	0	1	76	0	1	0	1
52	3	0	0	3	77	....	....	0	1
53	2	0	0	2	78	....	....	0	1

TABLE 18—*Continued*

Cow No.	Results with microscope		Results with animal test		Cow No.	Results with microscope		Results with animal test	
	Positive	Negative	Positive	Negative		Positive	Negative	Positive	Negative
79	....	....	0	1	1014	....	....	0	1
80	1	0	0	1	1017	...	...	0	1
83	2	1	1	0	Grade heifer	....	....	0	1
86	1	0	....	....	Small heifer	0	2	0	1
87	2	1	....	....	Large heifer	1	0	0	1
89	....	....	0	1	Lady Britton	0	2	0	2
95	....	....	0	1	Wild Eyes	0	2	0	2
97	1	1	....	....	Steer 650	0	2	0	1
98	1	0	....	....	Flower Strathmore	0	2	0	1
104	.	....	0	1	Oxford 2d steer	0	1	0	1
109	1	0	0	2	Oxford 2d cow	0	1	0	1
1002	....	..	0	1	Bull	0	1	0	1
1004	....	....	1	0	Columbia 11 (steer)	0	1	0	1
1007	...	....	0	1					
1008	....		0	1	Total	52	62	4	96

TABLE 19.—POST-MORTEM INSPECTION OF REACTING CATTLE

Cow No.	Location of tuberculous lesions
	(a) Cattle slaughtered and inspected at Urbana*
10	Bronchial lymph glands, lungs, liver, ovary, mesenteric lymph glands : generalized tuberculosis : condemned.
28	Bronchial and mediastinal lymph glands.
36	Lungs, mesenteric lymph glands.
37	Lungs, mediastinal lymph glands.
42	Lungs, bronchial lymph glands.
47	Heart, intestine, liver, and mesenteric lymph glands : generalized tuberculosis : condemned.
49	Lungs, bronchial lymph glands.
52	Lungs.
53	Retro-pharyngeal and inguinal lymph glands.
54	Liver, mediastinal and bronchial lymph glands.
56	Lungs, liver, bronchial and mesenteric lymph glands : condemned.
57	Liver, guttural, mediastinal, and bronchial lymph glands : condemned.
58	Large omentum, liver, guttural, mediastinal, and bronchial lymph glands : condemned.
77	Bronchial lymph glands.
86	Bronchial lymph glands.
87	Lungs, pleura, diaphragm, liver, mediastinal and bronchial lymph glands : generalized : condemned.
97	Lungs, bronchial lymph glands : generalized : condemned.

*We are indebted to the Dairy Department, University of Illinois, for the opportunity to make the post-mortem inspections upon the cattle slaughtered at Urbana, and for the records of inspection of the cattle slaughtered at Chicago, except for the last eight animals, the records of which have been furnished by the Animal Husbandry Department.

TABLE 19.—*Continued*

Cow No.	Location of Tuberculous lesions
	(b) Cattle slaughtered and inspected at Chicago.*
12	Liver, mediastinal lymph glands.
13	Nothing found.
15	Mesenteric lymph glands.
16	Lungs, mediastinal, bronchial, and mesenteric lymph glands.
19	Mediastinal lymph glands.
29	Nothing found.
33	Nothing found.
39	Bronchial lymph glands.
41	Retro-pharyngeal and mesenteric lymph glands.
51	Lungs, retro-pharyngeal and mesenteric lymph glands : condemned.
59	Parotid and mediastinal lymph glands.
60	Bronchial lymph glands.
61	Nothing found.
64	Retro-pharyngeal lymph glands.
66	Lungs, liver, bronchial and mediastinal lymph glands.
67	Nothing found.
71	Mediastinal lymph glands.
72	Mesenteric lymph glands.
76	Mediastinal lymph glands.
79	Nothing found.
80	Bronchial and mediastinal lymph glands.
83	Lungs, bronchial and mediastinal lymph glands.
95	Bronchial lymph glands.
104	Retro-pharyngeal and mesenteric lymph glands.
109	Non-tuberculous abdominal abscess.
1002	Lungs, bronchial and mediastinal lymph glands.
1004	Lungs, bronchial and mediastinal lymph glands.
1007	Bronchial lymph glands.
1008	Bronchial and mediastinal lymph glands.
1017	Lungs, bronchial and mediastinal lymph glands.
G.H.	Nothing found.
L.H.	Nothing found.
S.H.	Bronchial lymph glands.
L. B.	Nothing found.
W.E.	Nothing found.
S.6.	Nothing found.
F. S.	Nothing found.
O. S.	Nothing found.
O. C.	Nothing found.
B.	Nothing found.
C. S.	Nothing found.

*We are indebted to the Dairy Department, University of Illinois, for the opportunity to make the post-mortem inspections upon the cattle slaughtered at Urbana, and for the records of inspection of the cattle slaughtered at Chicago, except for the last eight animals, the records of which have been furnished by the Animal Husbandry Department.

may be much worse than the average. It is important to point out that even in the well-fed and apparently healthy herds, which have reacted to tuberculin, some animals do pass tubercle bacilli in the feces. That the general percentage is as high as indicated by the

work of some authors does not seem warranted when we consider the list of all the naturally tuberculous cows that have been proved by guinea pig inoculation to be passing virulent tubercle bacilli in their feces. This list comprises sixteen animals as follows:

SCHROEDER AND COTTON,

1. Cow No. 113—Bad advanced tuberculosis. Still alive.
2. Cow No. 373—Generalized tuberculosis at autopsy.
3. Cow No. 1—Generalized tuberculosis at autopsy
4. Cow No. 552—Still alive in good condition.

REYNOLDS AND BEEBE,

5. Cow No. 2½—Generalized tuberculosis at autopsy.

PETERS AND EMERSON,

6. Cow No. 102—No autopsy. The cow was poor but the symptoms of the disease were not marked. The number of acid-fast bacilli in the feces as revealed by the microscope was exceedingly large. In every field a number of them could be located, and in one place an area of degenerating tissue with many bacilli clinging to it was found.

7. Cow No. 20—Showed no sign of tuberculosis. Her feces contained fairly large numbers of acid-fast organisms. No autopsy.

8. Bull No. 204—Is of special interest because of his exceptionally fine appearance. This was a large Aberdeen Angus bull, remarkably fat and sleek. However, he had a persistent but moderate cough. No autopsy.

ROYAL COMMISSION ON TUBERCULOSIS, ENGLAND,

9. Cow B—Autopsy showed bad generalized tuberculosis.
10. Cow C—Autopsy showed bad generalized tuberculosis.
11. Cow A—Autopsy showed tuberculosis of intestine and lungs.
12. Cow D—When alive, fat and in good condition. Tuberculosis could be detected only by tuberculin test. Autopsy showed large tubercles in the lungs and bronchi.
13. Cow F—Autopsy showed severe generalized tuberculosis, apparently originating in the alimentary tract.

NEW CASES IN THIS REPORT.

14. Cow 83—Autopsy showed generalized tuberculosis.
14. Cow 1004—Autopsy showed tubercles in the lungs and bronchial lymph glands.
16. Cow 56—Autopsy showed tubercles in the lungs, liver, and mesenteric, and bronchial lymph glands.

It should be noted that of these 16 cows, that have been shown by guinea pig inoculation to be passing virulent tubercle bacilli in their feces, the eleven slaughtered were very badly tuberculous. Every one of the eleven showed abscesses of the lungs; seven, ulcers of the intestines; and six, tuberculosis of the liver. Every one of these surely should have been condemned at slaughter. Of the animals not examined *post mortem,* two were in advanced stage of tuberculosis and three in apparently good condition. The most important fact to be noted here is that a cow may be in an apparently good condition and yet be passing tubercle bacilli in her feces which will produce tuberculosis in guinea pigs. It appears

also that under natural conditions a cow does not pass virulent tubercle bacilli in the feces unless her lungs, intestines, or liver are affected with open tuberculosis. But this can not be ascertained from the outward appearance of the animal.

CONCLUSIONS

1. Microscopic tests show acid-fast micro-organisms in the feces of non-reacting as well as of cattle reacting to the tuberculin test.

2. Microscopic tests can not, in all cases, be depended upon to reveal virulent tubercle bacilli when they are present.

3. By guinea pig inoculations, tubercle bacilli in a suspension of fresh cow feces can be detected when present in an amount as small as one hundred billionth of a gram in the dose inoculated.

4. Reacting cows in an apparently good state of nutrition may be passing virulent tubercle bacilli in such quantities that one cubic centimeter of a two percent emulsion of their feces will produce generalized tuberculosis in guinea pigs.

5. Under ordinary conditions only a small percentage of tuberculous cattle are excreting tubercle bacilli in their feces at any given time.

6. At this Station, ninety-seven samples of feces from sixty-two tuberculous cattle have been tested for tubercle bacilli by inoculation of guinea pigs. Tubercle bacilli were found four times in samples from three animals.

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TUBERCLE BACILLI IN BLOOD OF TUBERCULOUS ANIMALS

LITERATURE

In the Tables 20, 21 and 22 giving the literature upon this subject two methods of determining the presence or absence of tubercle bacilli in the blood are contrasted, that of bacterioscopy and that of experimental inoculation. Calmette[11] has briefly summarized the bacterioscopic methods as follows:

"Bacterioscopy is the direct search for the tubercle bacillus in the juices of the organism or in the blood. It must be regarded as an exceptional method, because, even in the case of sero-pleural effusions, the bacilli are discovered at the most in two percent of the cases (Netter). It is so persistently negative in the case of blood that there is nothing gained by using it for the study of that fluid. The ingenious method of indirect bacterioscopy, known, since the work of Jousset, as inoscopy, in which the blood is centrifugated after the fibrin has been artificially digested or rendered incoagulable by means of various procedures (washing with soda, after Bezançon, Griffon and Philibert; the leech method of Lesieur), is so delicate in its application, and fraught with so many errors on account of the presence of other acid-fast, non-tuberculous bacilli, that its results cannot be interpreted with sufficient accuracy for clinical purposes. The same is true of other methods of bacterioscopy, based on a previous hemolysis of the red blood-cells (alcohol-hemolysis of Loeper and Louste, hydrohemolysis of Nattan-Larrier and Bergeron). At the beginning of a tuberculous infection the bacilli are so few in number in the blood and in serous exudates that there is no possibility of finding them in these fluids."

Rosenberger has since devised a modification of the direct bacterioscopic method, the essential features of which are sedimentation of citrated blood, smearing the sediment in thick layers on slides and laking the hemoglobin with distilled water before staining for tubercle bacilli. Uhlenhuth and Xylander[47] have employed "antiformin", a mixture of chlorinated soda and caustic soda, to dissolve the blood clot, and have examined the sediment of the resulting solution for tubercle bacilli, a method very similar in principle to that of Jousset. Schnitter has first laked the freshly drawn blood by mixing it with three percent acetic acid or with three percent citric acid. The washed sediment of this mixture was then digested with fifteen percent antiformin solution. The remaining sediment, very minute in quantity, was washed with water and then stained and examined for tubercle bacilli. None of these methods of bacterioscopy is so delicate as that of animal inoculation. By this method some of the suspected material either without special preparation or after treatment by some of the various methods given above is injected into test animals.

Table 21, "Tubercle bacilli in blood of animals injected with pure culture" shows that some of the results of each author quoted are positive by the animal inoculation test. Of the three authors giving results by both methods, Anderson has wholly negative results by the microscopic test, while his results by the inoculation test are positive in sixty-six and two-thirds percent of the cases. Anderson, also, obtained positive culture results by inoculating glycerine potato medium with the blood of these same animals.

TABLE 20.—TUBERCLE BACILLI IN THE BLOOD OF MAN

No.	Author	Date	Microscopic test				Animal inoculation test			
			Method	Cases	Positive	Percent	Cases	Positive	Percent	Remark
1	Weichselbaum[50]	1884	Direct smear	4	4	100	..	..	....	Acute cases
2	Weichselbaum[50]	1884	Direct smear	?	0	0	..	..	..	Chronic cases from dead body
3	Meisels[29]	1884	Direct smear	8	8	100	..	..	....	Acute cases from dead body
4	Meisels[29]	1884	Direct smear	1	1	100	..	..	....	Blood taken during life
5	Lustig[27]	1884	Direct smear	?	+	?	..	..	...	After long search
6	Rutimeyer[40]	1885	Direct smear	1	1	100	..	..	....	Patient just dead
7	Sticker[44]	1885	Direct smear	3	3	100	.	..	....	
8	Sticker[44]	1885	Direct smear	1	0	0	..	..	..	Made 80 preparations

TABLE 20.—*Continued*

No.	Author	Date	Microscopic test				Animal inoculation test			Remark
			Method	Cases	Positive	Percent	Cases	Positive	Percent	
9	Bezançon[5] Griffon and Philibert	1903	Homogenisation	2	2	100	..	..	..	
10	Jousset[21]	1904		...	...	....	35	11	31	Animal experiment
11	Bergeron[3]	1904	Inoscopy	26	0	0	26	1	4	Guinea pig test
12	Gary[17]	1904		...	...	....	35	3	14.3	Guinea pig test
13	Lesieur[22]	1904	Leech method	30	10	33.3	30	5	16.7	Guinea pig test
14	Loeper and Louste[25]	1905	Alcohol-hemolysis	3	0	0	..	..	....	
15	Ludke[26]	1906		.. .	...	....	3	3	100	Guinea pig test
16	Huguenin[19]	1908		...	.	.	1	1	100	Guinea pig test
17	Rosenberger[38]	1909	Rosenberger	300	300	100	?	?	?	Two cases positive by guinea pig test
18	Forsyth[14]	1909		12	10	83	..	..	...	
19	Rosenberg[37]	1909	Rosenberger	20	1	5	1	0	0	In the positive one, guinea pig inoculation failed
20	Webb[49]	1909	Rosenberger	?	0	0	..	..	...	
21	Stoll[45]	1909	Rosenberger	8	0	0	..	..	...	
22	Miller[30]	1909	Rosenberger	?	0	0	..	..	...	Preliminary report
23	Brown[8]	1909		...	...	....	13	0	0	
24	Bernstein[4] and Fried	1909	?	10	0	0	10	0	0	Guinea pig test
25	Anderson[1]	1909	Rosenberger	48	1	2	48	0	0	Guinea pig test
26	Liebermeister[23]	1909		...	...	..	50	20	40	Guinea pig test
27	Ravenel and Smith[36]	1909	Rosenberger	18	0	0	..	..	....	
28	Burnham and Lyons[9]	1909	Rosenberger	10	0	0	.	..	....	
29	Dailey[13]	1909	?	17	0	0	17	0	0	Animal tests
30	Petty and Mendenhall[35]	1909	Rosenberger	10	9	90	..	..	...	
31	Brem[7]	1909	Rosenberger	?	0	0	..	..	....	
32	Schnitter[42]	1909	Staubli technic and Antiformin	38	12	21	..	.	....	
33	Hewatt and Sutherland[18]	1909	Rosenberger	22	0	0	..	..	...	
34	Lippmann[24]	1909	Antiformin	25	11	44	..	..	...	
35	Tausig[46]	1909	?	?	0	0	?	0	0	
36	Foster[15]	1910	Rosenberger	18	14	78	..	..	...	
37	Burville-Holmes[10]	1910	Rosenberger	56	5*	9	37	0	0	Guinea pig test
38	Clifford[12]	1910	Rosenberger	?	+	?	..	..	....	
39	White and Avery[51]	1910	Rosenberger	51	0	0	47	0	0	Guinea pig test

*The author considers at least four of these five positive results to have been due to acid-fast bacilli in the distilled water used.

TABLE 21.—TUBERCLE BACILLI IN BLOOD OF ANIMALS INJECTED WITH PURE CULTURE OF B. TUBERCULOSIS

No.	Author	Date	Animal	Test positive after injection	Microscopic test				Animal inoculation test			Remark
					Method	Cases	Positive	Percent	Cases	Positive	Percent	
1	Villemin[48]	1865	Rabbit			...	...	....	(?)	+	(?)	Blood from femoral vein
2	Baumgarten[2]	1881	Rabbit			...	...	....	(?)	+	(?)	Generalized tuberculosis in the rabbit
3	Nocard[33]	1887	Rabbit	4 hrs.		...	...	....	(?)	+	(?)	Avian culture
4	Jeannel[20]	1888	Rabbit	7 days		...	...	....	7	6	85.7	Subcutaneous inoculations of tissues
5	Gaertner[16]	1893	Rabbit	5 hrs.		...	...	....	(?)	+	(?)	
6	Bergeron[3]	1904	Rabbit	7 days		...	...	....	4	2	50	
7	Nattan-Larrier & Bergeron[32]	1905	Rabbit	9 days	Hydro-hemolyses	10	9	9	7	4	57	Obtained sample by heart puncture; injected into ear vein. First to obtain (potato) cultures
8	Neumann and Wittgenstein[34]	1906	Dog	35 days		...	...	...	(8)	+	(?)	Sample from carotid into guinea pigs
9	Marmorek[28]	1907	G. pig	4 to 6 weeks		...	...	....	(231)	+	(?)	Animal injected in various ways; sample taken by heart puncture and from vessels
10	Anderson[1]	1909	Rabbit	27 days	Rosenberger	7	0	0	6	4	66	Tested by guinea pig inoculation and by culture on potato
11	Webb[49]	1909	Rabbit	(?)	Inoscopy	(?)	+	(?)	.....	..	....	
12	Webb[49]	1909	Rabbit	(?)	Rosenberger	(?)	0	0	.....	..		
13	Bongert[6]	1909	Rabbit	24 days		...	...	....	6	6	100	Animal injected intravenously; sample taken from carotid and femoral
14	White and Avery[51]	1910	G. pig	46 days	Rosenberger	1	1	100	1	1	100	Sample of heart blood
	Total					18	10	55.5	31	23	74.2	

In regard to the relative reliability of the two methods, most authorities hesitate to accept the results of mere microscopic examination unless these have been carefully compared with the results of animal inoculation. They all regard animal inoculation as an essential part of any absolutely reliable procedure for identi-

fying tubercle bacilli. Brem has called attention to the occurrence of acid-fast bacteria in laboratory distilled water as a prominent source of error in Rosenberger's method and this finding has been confirmed by Burville-Holmes. One should be very guarded in accepting the results of bacterioscopy. Animal inoculation itself is not free from chances of error, such as natural or accidental tuberculous infection and pseudo-tuberculous changes, but these errors have been guarded against in most of the published work and the results are correspondingly more reliable.

Table 20 shows a total of 800 cases tested by the microscopic method with 393 or 49 percent positive, and a total of 341 cases tested by animal inoculation with 44 or 12.9 percent positive. This percentage of positive cases as shown by microscopic test compared to the percentage of positive cases as revealed by animal inoculation test has no special significance unless it be to point out the likelihood of error in the former method. From the results given it is quite evident that tubercle bacilli do circulate at times in the blood of man. The results in Table 21 show that when pure cultures of tubercle bacilli are injected directly or indirectly into the circulating blood they may remain in the blood for some days in quantities large enough to infect guinea pigs. Here it is noted that the percentage of positive findings is much larger as would be expected. It is also noted that the percentage of positive findings for the animal inoculation tests is larger than the percentage for the microscopic tests upon the same samples of blood. Table 22 indicates that the tubercle bacilli have been found in the circulating blood of naturally tuberculous cattle by only one observer.

TABLE 22.—TUBERCLE BACILLI IN THE BLOOD OF CATTLE

No.	Author	Date	Microscopic test				Animal inoculation test			
			Method	Cases	Positive	Percent	Cases	Positive	Percent	Remark
1	Bongert[6]	1909		...	...	..	11	7	64	Rabbits and guinea pigs inoculated
2	Schroeder[43] and Cotton.	1909	Rosenberger..	42	0	0	42	0	0	Guinea pig test
3	Mohler[31].....	1909	Rosenberger..	8	0	0	8	0	0	Guinea pig test
4	Sawyer[41]	1909	Rosenberger..	21	0	0	21	0	0	Guinea pig test

EXPERIMENTAL WORK ON CATTLE

INSTRUMENTS USED IN COLLECTING SAMPLES

The apparatus used for collecting the blood of the cows consists of a small flask holding 75 cubic centimeters. (See Fig. 4). To this flask is fitted a rubber stopper thru which two glass tubes pass. To one of the glass tubes is attached a piece of small rubber tubing about eight inches long which holds a large syringe needle (lumen 0.5 mm); the other glass tube is plugged with cotton. This latter serves as an escape for the air as the blood flows in. In the flask were placed 25 cc. of one percent sodium citrate in 0.8 percent salt solution. This solution when mixed with an equal quantity of blood prevented the clotting for a number of hours.

TAKING THE SAMPLE

The cow was haltered and held by an assistant or tied to the stall. Her hind legs were tied together to prevent kicking or jumping. The blood was taken from the large udder vein. From this vein the sample was

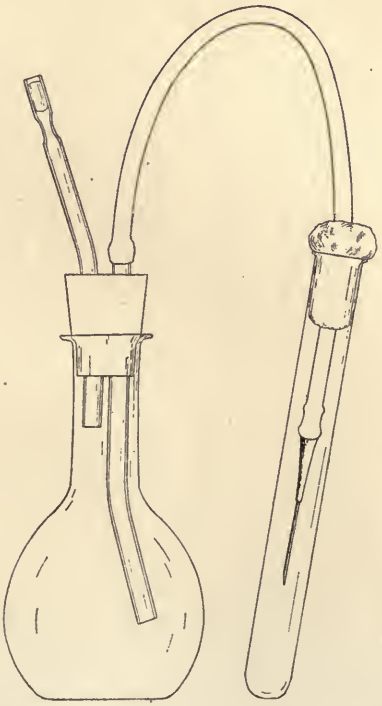

FIG. 4. FLASK FOR COLLECTING BLOOD SAMPLES.

procured with little difficulty and it is much more convenient than the jugular, ear, or leg vein, each of which was tried. The udder vein is better because it is large, prominent, and more easily punctured, and also because the cow, to move away from the operator, must move her whole body, while a vein on the head or leg is very much more easily jerked away. Frequently the udder vein could be punctured without any evidence of discomfort on the part of the cow.

The skin over the vein just in front of the udder was thoroly scrubbed and the hair shaved from a small area. A spot the size of a dime in this shaven area was cauterized with strong (95 per-cent) carbolic acid. This was allowed to dry for a minute or two. The cauterization not only disinfects but also anesthetizes the skin. The milk vein was grasped in one hand and the syringe needle first pushed thru the skin and then a little way from this opening pushed thru the wall of the vein. Abundant and rapid flow of blood was obtained, and this was mixed with the citrate solution by rotating the flask. As soon as a sufficient amount of blood had been obtained the needle was withdrawn and the vein

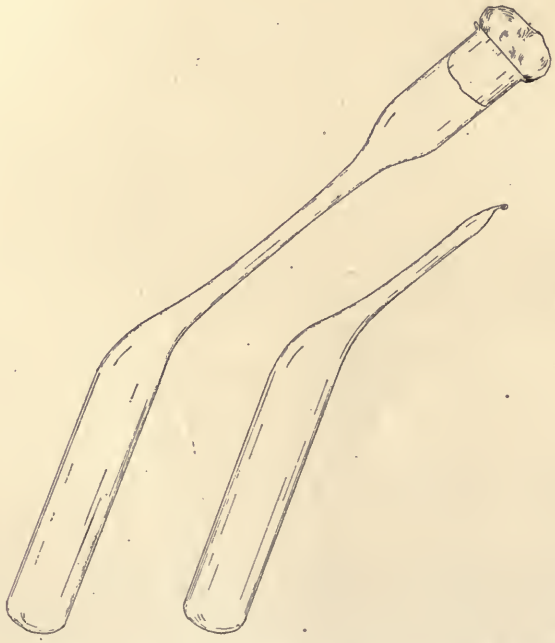

FIG. 5. SPECIAL CENTRIFUGE TUBE, BEFORE AND AFTER SEALING.

compressed for a minute at this place to prevent further loss of blood. The carbolic acid was then washed off from the skin with 95 percent alcohol. This operation of taking the blood has in our experience produced no injurious effect upon the cows at any time.

TESTING THE SAMPLE

The blood sample as soon as taken was well mixed with the sodium citrate solution and after bringing it to the laboratory ten cubic centimeters were put into a test tube and placed in the ice box for twenty-four hours. The sediment from this portion was stained by Rosenberger's method. Twelve samples from eight cows examined by this method gave always negative results. Of the remaining portion of the

TABLE 23.—TESTING THE BLOOD OF TUBERCULOUS CATTLE FOR TUBERCLE BACILLI

Cow No.	No. of test	Animal inoculation test					Microscopic test	
		Guinea pig number	Date of inoculation	Mode of death	Days after inoculation	Autopsy	Centrifuge sediment	Rosenberger method
10	1	583	9-10-'09	Killed	61	Normal	0	0
		584	9-10-'09	Killed	61	Normal	0	0
	2	610	9-16-'09	Killed	62	Normal	0	0
		611	9-16-'09	Killed	62	Normal	0	0
37	1	585	9-11-'09	Killed	61	Normal	0	0
		586	9-11-'09	Killed	61	Normal	0	0
	2	631	9-18-'09	Killed	60	Normal	0	0
52	1	608	9-15-'09	Killed	59	Normal	0	0
		609	9-15-'09	Killed	59	Normal	0	0
54	1	587	9-13-'09	Killed	57	Normal	0	0
57	1	596	9-14-'09	Killed	59	Normal	0	0
		597	9-14-'09	Killed	59	Normal	0	0
	2	612	9-16-'09	Killed	62	Normal	0	0
		613	9-16-'09	Killed	62	Normal	0	0
58	1	598	9-14-'09	Killed	59	Normal	0	0
		599	9-14-'09	Killed	59	Normal	0	0
	2	630	9-18-'09	Killed	60	Normal	0	0
Flower Strathmore	1	704	11-25-'09	Killed	39	Normal	0	0
		705	11-25-'09	Killed	39	Normal	0	0
Oxford 2d	1	706	11-25-'09	Killed	60	Normal	0	0
		707	11-25-'09	Killed	60	Normal	0	0

sample about 40 cc. was transferred to two specially prepared centrifuge tubes shown in Figure 5, by means of a Pasteur bulb pipette. These tubes were then hermetically sealed in the flame. The blood in the tubes was then centrifuged for 30 minutes at 2200 revolutions per minute. The capillary tip was then broken off, the opening flamed and the clear supernatant liquid pipetted off and discarded. To the sediment, consisting chiefly of blood corpuscles, sterile distilled water was added by means of a Pasteur bulb pipette. The tubes were again sealed in the flame, the con-

tents thoroly mixed, and again centrifuged. In this way the hemoglobin was dissolved out of the red blood corpuscles and removed in the supernatant liquid, while the sediment, consisting of red blood cell stroma, white blood cells and possibly bacteria, became colorless and small in quantity. After repeated washing with sterile distilled water (always with the aid of the centrifuge) the sediment was obtained as a tough, gelatinous, colorless mass. We consider this technic of special importance because by its use the bacteria contained in a very considerable quantity of blood may be concentrated into a very small volume and separated from the hemoglobin and the liquid portion of the original blood, constituents which are likely to interfere with further steps in testing for bacteria. Moreover, by this technic properly carried out, contamination by microorganisms from any other source may be absolutely excluded. A small part of this material was smeared on slides and stained for acid-fast bacilli, and the remainder injected into two guinea pigs.

RESULTS

The results of the microscopic examination and animal inoculation tests for tubercle bacilli in the circulating blood of cattle reacting to tuberculin are given in Table 23. The blood of eight cattle was tested for tubercle bacilli, four of them once and four of them twice. Six cows, Nos. 10, 37, 54, 57, 58, and 52, were from the dairy herd, and their autopsy records are given in Table 19. The other two, Flower Strathmore and Oxford the Second, were from the beef cattle herd and their autopsy records are given in Table 19, F. S. and O. S. Eighteen guinea pigs were inoculated with centrifuge sediment. All of them survived until chloroformed for examination. None became tuberculous. Every sample was tested microscopically by staining the centrifuged sediment and by staining the sediment after the Rosenberger method as previously described. In no case were any acid-fast organisms found.

CONCLUSIONS

1. At present the results of the different investigators in regard to the detection of tubercle bacilli in the circulating blood are conflicting.

2. This difference is probably due in part to the methods used. If larger quantities of blood are used for the sample injected into animals, as was done by Liebermeister in detecting tubercle bacilli in the blood of man, or by Bongert in detecting the tubercle bacilli in the blood of cattle, a larger percent of positive findings would be expected, as was the case with these authors.

3. Rosenberger's contention that tubercle bacilli are frequently found in the blood by microscopic test has not been confirmed by

other workers, and it is very probable that his results are largely erroneous.

4. As a diagnostic procedure the examination of the blood for tubercle bacilli is of little value.

5. Tubercle bacilli may undoubtedly circulate in the blood of man and animals, at times in large numbers. Whether they do frequently so circulate in very small numbers and how often they are likely to do so, is still unsettled.

6. In the circulating blood of tuberculous cattle, tubercle bacilli have been found by good investigators, but their occurrence here would appear to be rather infrequent. In our own tests the results were wholly negative, but it should be remembered that the cattle tested were not in an advanced stage of tuberculosis.

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PUBLIC HEALTH RELATIONS

IMPORTANCE OF HUMAN TUBERCULOSIS

Tuberculosis is the most serious disease of both men and domestic animals. The report of the Bureau of Census, 1907, showed 11.2 percent of all human deaths to be due to this disease. In that year there were 200,000 deaths from tuberculosis in the United States. In Illinois the percentage of deaths from tuberculosis appears to be slightly higher than in the United States as a whole, being recorded as 11.49 percent of the deaths from all causes. Human tuberculosis appears to be decreasing slightly, but among the food producing animals, on the other hand, there is an increase in the amount of this disease.

POSSIBILITY OF HUMAN INFECTION WITH THE BOVINE BACILLUS

Smith[6] has estimated that about one percent of cases of human tuberculosis are due to tubercle bacilli of the bovine type. Fibiger[2] says that, according to the summary of figures published in the last few years from some of the hospitals of Kiel, Berlin, and Copenhagen, about five percent of all the children that come to autopsy are affected with primary bovine intestinal tuberculosis. Park and Krumwiede[5] found, in an examination of 435 cases of tuberculosis, 32, or 7.4 percent, caused by tubercle bacilli of the bovine type. In adults over 16 years of age only one case of the 297 examined was caused by the bovine bacillus; while in children between the ages of 5 and 16 years, nine out of 54; and in children under five years of age, twenty-two out of a total of 84 cases examined, or 26.2 percent, were found to be caused by the bovine type of tubercle bacillus. In the series of children from the Babies' Hospital, in which the best average conditions obtained, four out of 43, or 9.3 percent, of the fatal cases of tuberculosis among children under 5 years of age were due to bovine tubercle bacilli; while in the series from the Foundling Hospital, consisting almost exclusively of children fed upon cow's milk, five in 9, or 55 percent, of the cases of tuberculosis under six years of age were affected with the bovine tubercle bacillus. These authors have compiled the authentic cases in the literature, and combining these with the cases

found in their own examinations, they make a total of 1042 cases, of which 101, or 9.7 percent, were affections due to the bovine type of tubercle bacillus. In adults over 16 years of age nine out of 686 cases, or 1.3 percent; among children from 5 to 16 years of age thirty-three of 132 cases, or 25 percent; while among children under 5 years of age fifty-nine of 220 cases, or 27 percent, were due to the bovine bacillus.

That man may become infected thru the consumption of tuberculous food products is no longer doubted by the best scientists. In general, animals are more susceptible to the bovine type of tubercle bacillus than to the human type, and that man is an exception to the rule there is no good reason to expect. Many authentic cases in which man has become infected with tubercle bacilli of the bovine type have been cited, and it is clearly evident that children are much more liable to such infection than adults.

OPPORTUNITY FOR HUMAN INFECTION WITH THE BOVINE BACILLUS

The opportunity for man to become infected with bovine tubercle bacilli is almost exclusively thru the consumption of tuberculous food products such as butter, milk, and meat. By consulting Tables 1, 2, 4, and 5, one may get an estimate of the percentage of butter and milk, containing tubercle bacilli, that is sold upon the market. The percentage for butter is about 13.2; that of milk, 6.8. The discussion relating to tubercle bacilli in these two products has been given (see pages 327 and 333. The danger of infection from meat is not so great as from butter or milk for two reasons. First, children are more commonly infected thru the ingestion of tubercle bacilli in food products than are adults; and children eat less meat and more butter and milk. Second, tubercle bacilli if present in meat are usually killed by cooking. However, dried and smoked meats, and meats not thoroly cooked may contain virulent tubercle bacilli. Some information concerning the amount of tuberculous meat can be obtained from the reports of the Federal meat inspection carried on in the large cities (see page 407). While in the larger cities excellent work is being done to prevent the selling of tuberculous milk, butter, meat, and other foodstuffs, there is great need to extend this work to the smaller cities and more generally thruout the country.

CAN A CITY ENFORCE A TUBERCULOSIS ORDINANCE

Municipalities should be giving attention thru their Boards of Health to the inspection of food products, especially butter, milk, and meat. Most large cities have milk ordinances and various pure food ordinances. The question has been raised as to whether the Federal or State Constitutions are violated

by enforcing ordinances prohibiting the sale of milk, or milk held, kept, offered for sale or delivered without a permit from the Board of Health, and this question has been decided by the courts. Such a case is reported in 175 N. Y. 440, The People of the State of New York ex rel. Simon Lieberman, appellant, vs. John E. Vandecarr as Warden of the City Prison of the City of New York, Respondent. The question in this case was whether Simon Lieberman could be forced to obtain a permit of the Board of Health of the City of New York before he could vend or offer for sale milk on the streets of said city. The court decided that the making and enforcing of such regulations is constitutional and within the powers of the city. The court said further, "In great cities, where, in certain sections, life exists under crowded conditions that can not be fully comprehended unless seen, and where many articles for table consumption by all classes of the community are liable to pass thru processes and conditions little short of appalling unless regulated by law, the full and rigorous exercise of the police power in the interest of the public health and general welfare is absolutely essential." This was the decision of the Supreme Court of the State of New York. The case was subsequently taken to the Supreme Court of the United States, October term, 1906, where the decision was affirmed.

HOUSE JOINT RESOLUTION NO. 20

Our legislature in 1909 passed House Joint Resolution No. 20, in which it questions the right of the city of Chicago to enforce certain ordinances regulating the sale of milk, butter, and cheese within said city. They also in this resolution question the efficiency of the tuberculin test as indicating tuberculosis in cattle, and the practicability of pasteurizing milk. The decision of the courts of New York concerning the first question has been cited. The reliability of the tuberculin test as shown by slaughter inspections is 98.37 percent according to the report by Melvin, already cited, whose authority has not been disputed. As to the pasteurization of milk the University Dairy here has for months been pasteurizing all the cream used in making butter. The quality of the butter produced by this dairy is considered by our local dealers to be the best upon the market and commands the highest price. Herr[2] says that the pasteurizing of cream at 75° to 90° C. has no detrimental influence on the quality of the butter. The taste of "cooked cream" does not go over into the butter. Five seconds heating at 85° C. is sufficient to pasteurize and in practice 85° C. for two minutes gives the best results. As is well known, the highly prized Danish butter is invariably made from pasteurized cream.

THE NECESSITY FOR POPULAR EDUCATION CONCERNING TUBERCULOSIS

To enforce successfully any public health regulations, not a few but a majority of the people must be informed upon the questions involved. Before permanent progress can be made in eradicating tuberculosis, it will be necessary to educate the whole people concerning this disease. It will be especially important for them to know its extent among men, and farm animals; to know its manner of transmission, the means of detecting its presence and the ways of handling the disease. One should know the danger from infected foods, the necessity of health regulations concerning the sale of foods likely to be contaminated with tubercle bacilli. The farmer especially should understand the danger both to his herd and to the people consuming the products of his herd when tuberculosis is found among his cattle.

SUMMARIZED STATEMENT

1. There are 200,000 deaths annually in the United States from tuberculosis of which it is estimated that about 92 percent are caused by the human type of tubercle bacilli, and 8 percent, or 16,000 cases, are caused by the bovine type.

2. In the latter cases the disease is probably contracted almost entirely thru the food and is found almost wholly among children.

3. There is a great need of extending the excellent sanitary regulations of some of our large cities to the smaller cities and thruout the country generally.

4. A system of popular education concerning tuberculosis, the tuberculin test, and health regulations would be of great benefit to the state.

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DISTRIBUTION OF TUBERCULOSIS AMONG FARM ANIMALS

INTRODUCTION

We know that tuberculosis is wide spread and very common among farm animals, and facts concerning its distribution are accumulating rapidly; yet there can not be said to be anything like adequate knowledge concerning this subject. Published reports upon the results of the tuberculin test are available for only a fraction of one percent of the cattle in the United States. In Illinois comparatively little testing has been done, and until this is done we can not know definitely concerning the amount of tuberculosis in this state. Hughes[5] says, "Illinois, unquestionably has an average of 25 percent of all dairy animals infected with tuberculosis". He says further that examinations of herds in State institutions have given a still higher percentage. From all we know it may be far more than an average of 25 percent for dairy cattle, but until the state has records of tests on a large portion of the dairy cattle it can have no very definite knowledge upon this point.

MEANS OF ASCERTAINING DISTRIBUTION

There are three means of ascertaining the distribution of tuberculosis among farm animals, (1) laboratory tests of butter, oleomargarine, and milk for tubercle bacilli, (2) tuberculin testing of cattle, and (3) government meat inspection. The reports of veterinarians and farmers are of some value in determining distribution, but we have no detailed reports from this source of information.

LABORATORY TESTS

Laboratory tests of dairy products are indeed not at all a definite means of determining distribution, but from the Tables, No. 1, No. 2, No. 4, and No. 5, there can be obtained considerable information concerning the distribution of tuberculosis among dairy cattle. In this country too little work has been done on the determination of the presence of tubercle bacilli in market butter to be of value in this connection. From the reports of work in Germany, it is likely that nearly 10 percent of all their market butter contains tubercle bacilli virulent to guinea pigs. We have reason to think

that it would be as high in this country since Table 3, *Bacillus Tuberculosis* in Market Milk, is even higher than that shown for the presence of *Bacillus tuberculosis* in the market milk of Germany. The average of the positive findings of the three largest series of tests made for tubercle bacilli in market milk in this country is 17.5 percent. We refer to the work of Anderson,[1] Hess,[4] and Field,[3] of Washington, New York, and Louisville, respectively. Their total samples examined aggregate 447 with a total of 78 samples containing tubercle bacilli.

TUBERCULIN TESTING OF CATTLE

The reports of tuberculin testing give the most accurate information we have concerning the distribution of tuberculosis among dairy cattle. This however is very inadequate when we consider what a minute fraction of the total cattle have been tested. The Bureau of Statistics[7] of the Department of Agriculture has estimated for January 1, 1908, that there is a total of 71,267,000 cattle in the United States; and the Bureau of Animal Industry[7] has recorded for the last fifteen years (1893 to July 31, 1908 inclusive) the testing of 400,008 cattle, i. e., the number of tests in fifteen years is about 0.5 of one percent of the number of cattle in the United States in the single year 1908. This testing has been done irregularly, sometimes upon requests by owners, at other times because cities made it compulsory that all cows supplying milk to the city should be subjected to the tuberculin test. Tho inadequate, it is the best means at hand for determining the distribution of bovine tuberculosis in the country. Of the 400,008 cattle tested as stated above, 37,000 reacted to tuberculin, or 9.25 percent. And from this Melvin says it is probable that 10 percent of the dairy cattle and 3.5 percent of all cattle in the United States have tuberculosis.

MEAT INSPECTION STATISTICS

There is given here a table from Melvin's paper cited above: Animals slaughtered under Federal Inspection in the United States with number and percentage found tuberculous during fiscal year 1908:

Kind	Number slaughtered	Number of animals condemned for tuberculosis	Percentage condemned for tuberculosis
Cattle.....	7,116,275	68,395	0.961
Calves	1,195,487	524	0.026
Hogs	35,113,077	719,309	2.049
Sheep.....	9,702,545	40	0.000
Goats.....	45,953	1	0.000
Total	53,973,337	788,269	1.460

Melvin says that the Federal meat inspection now covers more than one-half of all the animals slaughtered for food in the United States. So the above table gives a fairly good idea of the extent of the disease among the meat producing animals of the country.

In the State of Illinois and most of the other states in the Union there is no meat inspection except in the large slaughtering houses. So if the parties having animals for slaughter suspect that their animals are tuberculous, the tendency may be to sell them where there is no inspection. For this reason the animals slaughtered without inspection are likely to have a higher percentage of tuberculosis, and it is therefore quite certain that over one percent of all beef cattle and over two percent of all swine slaughtered in the United States are seriously affected with tuberculosis.

HIGH PERCENTAGE IN PARTICULAR INSTANCES

In some particular instances in several of the states, herds have been found to have a high percentage of tuberculous animals. In 1901, Russell and Hastings[14] reported the testing in Massachusetts of 24,685 cattle of which 12,443 or 50 percent reacted to tuberculin. In 1905, Russell[13] reported work done in 1903 and 1904, in which 21 herds in Wisconsin having wide spread infection were tested. These 21 herds aggregated 933 cows of which 518 or 55 percent were tuberculous. In 1901, Pearson and Ravenel[17] tested twelve herds having a total of 599 cattle, 484 or 80 percent reacting. In 1908, Ward and Haring[16] reported the testing of 22 herds in California near San Francisco totaling 1022 cows of which 326 or 31.9 percent reacted. In 1908, Moore[8] compiled figures obtained from work done under the direction of the Commissioners of Agriculture of New York State and of private veterinarians, and found 32.26 percent of the cows reacting to the tuberculin test.

DISTRIBUTION IN FOREIGN COUNTRIES

Denmark.—Bang[2] reported the tuberculin testing from 1898 to 1904 of 40,624 head of cattle. Of the calves under six months 12.1 percent were tuberculous; of the yearlings (6 to 18 months) 27.5 percent; of cows 1½ to 2½ years, 38.6 percent; of those 2½ to 5 years, 44.9 percent; and of those over 5 years old 48.0 percent were tuberculous. *Belgium.*—Heymans[6] said that there are about 2,000,000 cattle in Belgium of which about 1,000,000 are milk cows. About 40 percent of the cows and 10 percent of the other cattle or an average of 25 percent of all cattle have tuberculosis. *Germany.*—Ostertag[10] in 1907, recorded the testing of 215,837 dairy cattle in Saxony of which 115,100 or 43.6 persent reacted to tuberculin. Nocard[9] estimated that 25 percent

of all dairy cattle in the regions of Brie and Beance in France were tuberculous, while some other regions were free from this disease. The Royal Veterinary College of England[12] in 1900, reported the testing of 20,930 animals of which 5441 or 26 percent reacted.

There are some parts of the world, as for example, Japan, the northern parts of Norway and Sweden, the Steppes of Russia, the Island of Jersey, and parts of Africa and South America where bovine tuberculosis is entirely absent or very rare.[15] These are places where the importation of cattle has been small in amount and this disease has not yet been disseminated.

FACTORS INFLUENCING DISTRIBUTION

EXCHANGE OF CATTLE

The factors that are continuing to extend tuberculosis among farm animals are extensive trading in cattle on the one hand and the subtleness of the disease on the other hand. In order to improve the herds thoroughbred stock has been imported and incidentally tuberculosis has been introduced with it. Salmon[13] says, "Unfortunately, the breeders of Great Britain were not as skillful in avoiding tuberculosis as they were in increasing the size, perfecting the form, and hastening the maturity of animals, and the result has been not only that they unwittingly propagated the disease, but that they distributed it in the most *extensive* manner". The improvement and increase in transportation facilities, together with the wider recognition of the value of a few pure bred animals in improving the quality of a herd, have led to extensive buying and selling of cattle for breeding purposes. This trade in cattle is very essential to the welfare of the live stock industries, but it should be clearly recognized that precisely this is the chief mode of introducing tuberculosis into a previously healthy herd. The owner of a healthy herd cannot be too careful in selecting the animals which he expects to add to it. It is the nature of this disease to disguise its presence. The cow with the disease looks well, may breed well, eat well, and continue to give milk. In fact, it may be only in the last stage of the disease, long after she has become dangerous by distributing the germs in her feces, urine, or milk, that physical signs of tuberculosis appear. Thus it often happens that a whole herd may become infected before the owner recognizes the presence of the disease.

STOCK SHOWS AND FAIRS

Stock shows, state and county fairs are other means of disseminating the disease. At such a fair where the stock drink from the same watering trough, where in the show ring they may nose each other, a bad tu-

berculous cow may infect the stock of a number of other farms and the fact may not be discovered for months or years afterward. Stock owners ought to exercise great caution in taking their stock to such shows. No stock should be permitted to be shown unless the owner has a certificate of a negative tuberculin test for the stock he is entering. Laws were passed in Denmark seventeen years ago to prevent tuberculous cattle and swine from entering stock shows and fairs and these laws have materially lessened tuberculosis in Denmark.

HOGS FOLLOWING CORN-FED CATTLE

The practice of having hogs follow corn-fed cattle is another great means of distributing tuberculosis. It has been noted for some years that hogs shipped from the dairy regions, both in this and in foreign countries, have upon slaughtering far more tuberculosis than hogs from other parts of the country. This is due to the fact that hogs readily take tuberculosis by eating corn out of manure or by drinking the milk of tuberculous cattle. Hogs should never be permitted to follow tuberculous cattle. Calves and hogs should not be fed separator milk from the creameries unless the milk has been pasteurized or boiled.

VENTILATION AND OUT-DOOR LIFE

It is well known that living out of doors and getting an abundance of pure air tends to check and even to arrest tuberculosis in human beings. This may also be true among cattle. But it does not follow from this that if cattle live out of doors entirely, they will be entirely free from tuberculosis. Ward and Haring[16] reported the testing of 1022 cattle in California of which 326 or 31.9 percent were tuberculous, while tests in New York State compiled by Moore showed a percentage of 32.26 having this disease. In New York the cattle are more or less housed in barns thruout the year while in California the cattle tested by Ward and Haring were kept almost exclusively out doors in large pastures or ranges and only put in open sheds at milking and feeding time. The herds were otherwise handled much like the Eastern herds, the cattle were associated together at feeding times, and the herds were built up by "buying in" fresh cows. This indicates that the climate is not a very important factor in preventing the spread of tuberculosis in cattle. Whatever the climate, or the breed, or the stock, or the vigor, when tuberculosis is introduced by purchase or otherwise, unless means are taken to arrest its spread, it will finally infect and destroy the whole herd.

SUMMARY

1. Tuberculosis among farm animals is found extensively thruout the whole world.

2. Our knowledge concerning distribution is as yet very incomplete.

3. The three important means of ascertaining the distribution of tuberculosis among farm animals are thru the reports of laboratory tests of dairy products, tuberculin testing of cattle, and government meat inspection.

4. In particular instances the percentage of tuberculous cattle are very high, in some states ranging from 32 to 80 percent.

5. The percentage of tuberculous cattle in foreign countries where much more testing has been done than in the United States ranges from 25 to 48 percent.

6. The two things most favoring the distribution of this disease are the extensive trade in (tuberculous) cattle; and the difficulty of recognizing the disease by physical signs until it is far advanced.

7. The tuberculin test should always be applied before adding a newly purchased animal to the herd.

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METHODS OF COMBATING ANIMAL TUBERCULOSIS

Recognition by Physical Means

The first essential in combating tuberculosis of farm animals is the recognition of the disease. In general this cannot be done by a physical examination alone. No expert can recognize tuberculosis, except in the advanced stages, by making a physical examination ever so carefully. In advanced cases or in moderately advanced cases a cough is often present. In tuberculosis of the udder an emaciated quarter or knots in the udder may be noted. In the last stages of the disease, generally only shortly before death, the animal becomes much emaciated and then the condition may be recognized from the external appearance.

Recognition by Means of the Tuberculin Test

The tuberculin test is by far the best means of recognizing tuberculosis in cattle in its early stages. It is made by first ascertaining the normal temperature of the cow, then injecting a dose of tuberculin under the skin of the neck and then observing the effect upon the temperature produced by the tuberculin injected.

TUBERCULIN

Tuberculin is the product of an extensive growth of *B. tuberculosis* in an artificial medium. The medium used is six percent glycerine meat broth. This medium is made and sterilized in broad bottomed, cotton stoppered flasks. These flasks are inoculated with a pure culture and are kept at body temperature (37° Centigrade) for some weeks until a thick

crumpled membranous growth covers the surface of the glycerine broth. The bacteria are killed by heat and then removed by filtering thru a fine clay filter. The filtrate is then placed in an evaporating dish and concentrated to one-tenth its original volume. This concentrated filtrate is tuberculin. Tuberculin as just described is prepared by the Bureau of Animal Industry of the United States Department of Agriculture and is diluted with weak (one percent) carbolic acid to eight times its volume before use. In this form, it is more convenient for use than the original thick syrupy liquid. It is sent out free of charge in either the concentrated or in the diluted form ready for use.

OBSERVATION OF THE TEMPERATURE

The cattle to be tested should be kept in the stable, resting quietly for some hours at least before the temperature is taken. Disturbing factors such as excessive feeding, drinking large amounts of cold water, or excitement of the animal should be avoided. The temperature is taken with a self-registering physician's clinical thermometer, placed in the rectum of the animal. It is necessary to throw down the column of mercury in such an instrument each time it is used, and the position of the column should be carefully examined each time just before insertion of the thermometer, to be certain that the mercury is down below 99° Fahrenheit. Neglect of this precaution may lead to very serious error. The thermometer is tied with a string around the constriction and then fastened to the cow with a hair clamp or tied around the tail with a string so that if the thermometer falls it will not strike the floor and break. Before inserting the thermometer it should be washed in 5 percent carbolic acid, wiped with a sterile cloth and smeared with vaseline. It is then carefully inserted into the rectum for its whole length, where it is allowed to remain for three to five minutes. Two persons working together, one reading, the other recording the temperatures, can keep in use five or six thermometers at one time.

A cow's normal temperature is 101° to 102.5° Fahrenheit; if because of any fever, being in heat, calving, or something else, the temperature is abnormal the tuberculin test should not be made at this time. Such animals should be excluded from the test and should be tested at a subsequent time when the cause of the abnormal temperature is no longer present. A good method of procedure is, on the first day, to determine the normal temperature of the cow by beginning at eight o'clock in the morning and taking her temperature every two hours until six o'clock in the evening. The average of these temperatures may be regarded as her normal temperature. Excluding all cows with abnormal tem-

peratures, inject the tuberculin into the others at 8 p. m., and begin again the next morning at six o'clock and observe the temperature at two hour intervals or more frequently thruout the day. By comparison of the temperature on the day following the injection of the tuberculin with the temperature on the previous day the result of the test is determined. For the correct interpretation of the test a man of training, experience and judgment is necessary.

INJECTING THE TUBERCULIN

For the injection of the tuberculin a good syringe with several sharp stout needles is required. The needles may be sharpened from time to time on an oil stone. The syringe should hold from five to ten cubic centimeters and should be graduated in cubic centimeters. It should be sterilized by boiling before use. The injection is made subcutaneously in the neck. Here the skin is thin and is easily pulled into a fold. The needle, if sharp, can be pushed in with but little trouble. It should be held almost parallel to the surface of the neck, so as not to enter the muscles. Have the set screw placed so that the exact amount can be injected. For an animal weighing a thousand pounds two cubic centimeters is the usual dose; for animals weighing more or less, a proportionally greater or smaller amount of tuberculin should be used.

POSITIVE AND NEGATIVE REACTION

Usually in the positive reactions the maximum temperature on the day following the injection is from 3° to 5° Fahrenheit above the normal temperature; if the rise is less than 2°F. it is to be considered doubtful. Some consider 1.5° F. above the normal individual temperature as determined on the previous day as sufficient to indicate a positive reaction. The elevation of temperature should come on gradually, remaining practically at its maximum for a few hours, and gradually subside. A sudden elevation which is not continued is not to be considered a reaction. All doubtful cases should be retested after six to eight weeks. On the opposite page are some examples of temperature observations in typical positive and in typical negative cases.

RELIABILITY OF POSITIVE REACTIONS

The positive reactions of the tuberculin test are very reliable. The Bureau of Animal Industry reports tests from 1893 to 1908, upon 400,000 cattle; 24,784 of the reacting cattle were slaughtered and of these, 24,387 or 98.39 percent were found to be tuberculous. In Pennsylvania 4000 animals that had given characteristic reactions were slaughtered and examined, and the presence of the disease was demonstrated in all but eight of these animals, 99.8 percent. Fifty-eight of the cattle used in the ex-

TABLE 24.—TEST OF DISEASED HERD, MAY, 1905
(From New York Agr. Exp. Sta. Bull. 279)

Animal	Breed	Age	Weight	Temperature before injection				Tuberculin injected 10:00 P. M.	Temperature after injection					Reaction
				10:30 A. M.	2:30 P. M.	4:00 P. M.	8:15 P. M.		7:00 A. M.	9:45 A. M.	11:00 A. M.	12:15 P. M.	3:15 P. M.	
Chloe	Mixed grade	6	1100	101.3	102.2	101.6	101.4	2.2cc.	103.4	105.2	104.3	102.5	101.8	+
Lady	Jersey	11	960	101.8	101.4	102.0	100.7	1.9cc.	102.3	104.0	104.7	104.1	102.7	+
Kate..	Jersey grade	6	900	101.4	101.6	101.3	100.7	1.8cc.	101.6	101.3	101.3	101.8	101.8	—
Millie D ...	Jersey	13	950	101.2	101.1	100.9	101.2	1.9cc.	101.6	101.8	101.6	101.2	101.9	—
Lily.....	Jersey grade	6	800	100.6	100.9	101.6	100.6	1.6cc.	102.0	103.1	101.8	102.0	102.2	?

TABLE 25.—TUBERCULIN TEST CHART
(From records of the Illinois State Veterinarian, by courtesy of Dr. J. M. Wright)

Animal	Breed	Age	Weight	Temperature before injection			Tuberculin injected 9:00 P. M.	Temperature after injection					Reaction
				1:00 P. M.	4:00 P. M.	7:00 P. M.		7:00 A. M.	10:00 A. M.	1:00 P. M.	4:00 P. M.	6:00 P. M.	
Red and White	Mixed	6	1000	101.5	101.5	101.5	2 cc.	105.0	105.2	105.2	105.0	104.5	3.7° +
Roan	Mixed	8	1050	102.0	102.0	102.0	2 cc.	106.5	106.0	106.0	106.0	105.5	4.5° +
Red (1)..	Mixed	7	1050	102.0	102.0	102.0	2 cc.	102.0	101.5	101.5	101.5	.....	—
Red (2)..	Mixed	6	950	101.7	101.5	102.0	2 cc.	101.5	101.2	101.2	101.2	....	—

TABLE 26.—TUBERCULIN TEST CHART
(From records of the Dairy Department, University of Illinois, by courtesy of Professor C. C. Hayden)

Animal*	Age	Weight	Temperature before injection					Tuberculin injected 8:00 P. M.	Temperature after injection						Reaction
			5:30 A. M.	8:00 A. M.	11:00 A. M.	3:00 P. M.	7:00 P. M.		5:00 A. M.	7:30 A. M.	11:30 A. M.	2:00 P. M.	4:30 P. M.	8:00 P. M.	
1004	6	1100	100.6	101 0	101.6	100.6	101.9	2 cc.	103.2	106.3	104.8	104.9	104.7	101.9	+
1007	8	1250	101.3	101.0	101.2	102.0	101.2	2 cc.	104.3	105.3	105.7	106.3	105.2	102.2	+
1015	7	1100	100.8	101.1	101.6	101.6	101.1	2 cc.	101.4	101.6	101.4	101.3	102.6	101.2	—
1018	8	1019	100.8	100.9	101.5	102.1	100.8	2 cc.	101.2	101.6	101.3	101.3	102.2	101.7	—

*These animals were all grade Holsteins.

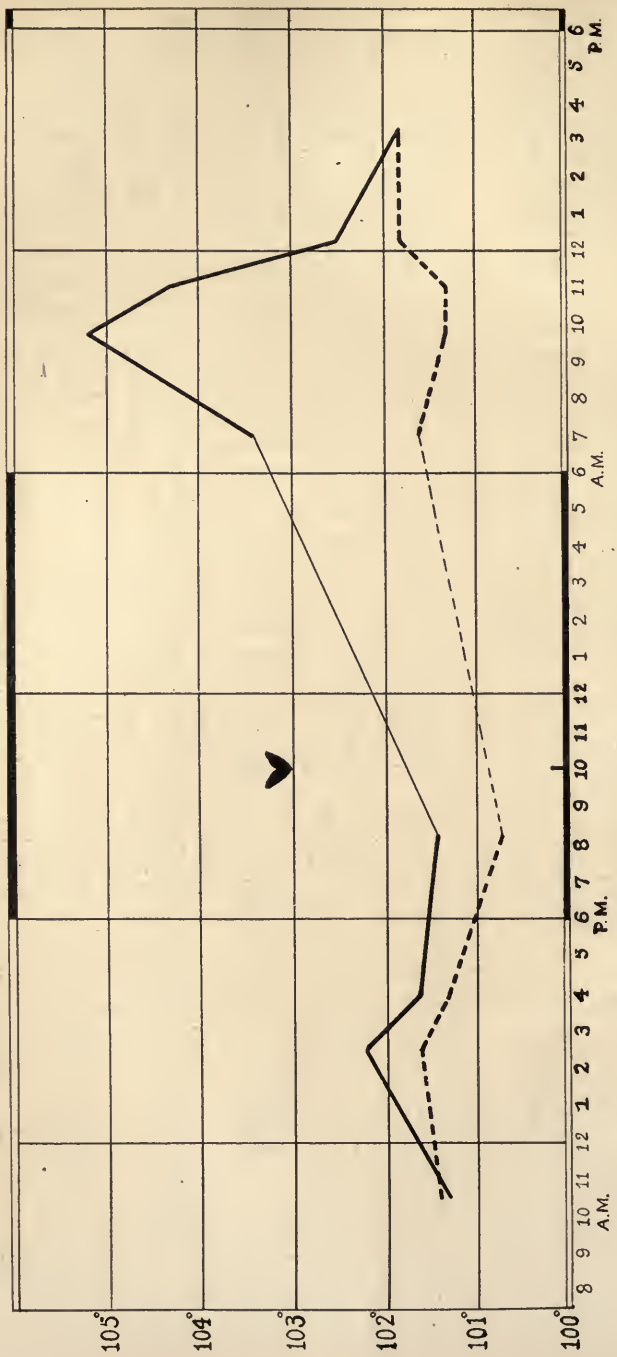

FIG. 6. TUBERCULIN TEST. TIME OF INJECTION, 10 P. M.
POSITIVE REACTION (CHLOE) ————
NEGATIVE REACTION (KATE) - - - - - -
(Data from New York Agr. Exp. Sta. Bull. 277.)

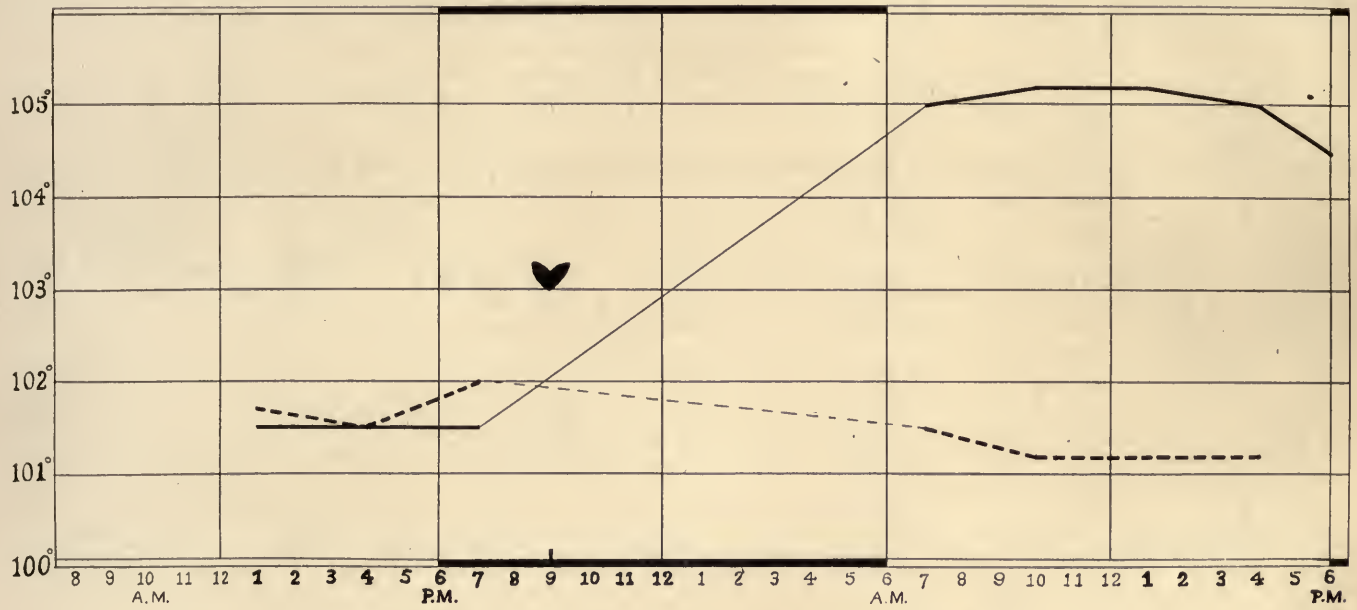

FIG. 7. TUBERCULIN TEST. TIME OF INJECTION, 9 P. M.
POSITIVE REACTION (RED AND WHITE) ——————
NEGATIVE REACTION (RED NO. 2) - - - - - - -

(Data from the records of the Illinois State Veterinarian, by courtesy of Dr. J. M. Wright.)

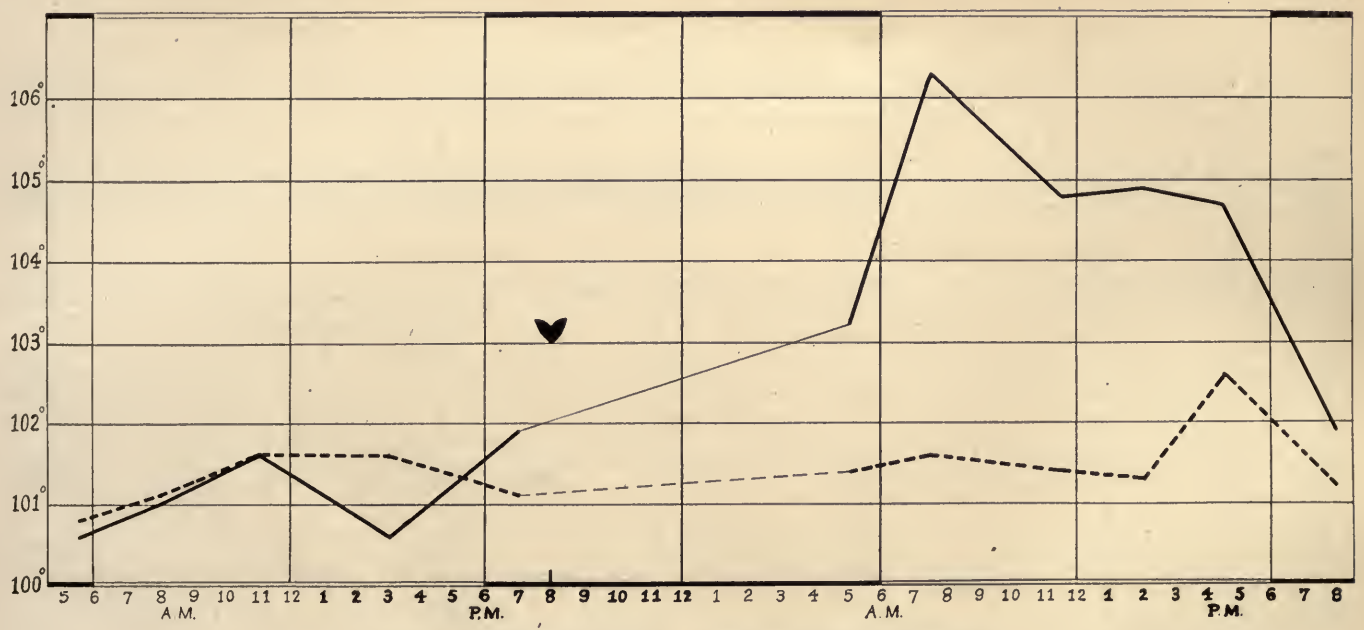

FIG. 8. TUBERCULIN TEST. TIME OF INJECTION, 8 P. M.
POSITIVE REACTION (1004) ——————
NEGATIVE REACTION (1015) - - - - - -

(Data from the records of the Department of Dairy Husbandry, University of Illinois, by courtesy of Professor C. C. Hayden)

perimental work of this bulletin that had given positive tuberculin reactions have been slaughtered. In seventeen of them, slaughtered and inspected by us in Urbana, typical lesions of tuberculosis were found in every case. Of the forty-one slaughtered and inspected at Chicago, lesions of tuberculosis were found in only twenty-four. The work of the government inspectors is necessarily done rapidly, and it is not impossible that slight lesions, unimportant from the standpoint of food value of the carcass, might be overlooked.

The tuberculin test is not an absolutely reliable test for the presence or absence of tuberculosis. It is, however, *very reliable when positive;* not so reliable when negative. It is by far the most reliable means known to determine the presence of tuberculosis.

NATURE OF THE REACTION

A consideration of the nature of the reaction as explained by Theobald Smith[3] may do much to clear up the confusion regarding the failure to react which sometimes occurs in tuberculous animals. The tubercle bacilli growing in the body induce certain tissue changes and arouse certain new functions of the tissues which are in the nature of a partial immunization. It is the action of the specific product, resulting from these changes, upon the tuberculin that causes the latter to split off a poisonous substance, which poison acting upon the tissues causes the elevation of temperature. Now, when the lesion is healing there is formed about it a fibrous wall which tends to isolate it from the surrounding tissues. This wall prevents the specific product of the tissue changes, induced by the tubercle bacilli, from getting outside it, and thus, not being able to act upon the tuberculin, it fails to split off the poisonous product, and no elevation of temperature occurs. Therefore in a cow with only a few healing lesions a reaction may not occur. But such a lesion may afterward become active and so give rise to a positive reaction at a later time. Harding and Smith[4] have described a cow (Millie D.) which had reacted and then failed to react for three years. At her death, they found a few tubercles, no larger than a pea and completely encapsulated. These tubercles, crushed in sterile water and injected into guinea pigs, produced generalized tuberculosis.

The period between infection and the time when a tuberculin reaction will first take place is known as the period of incubation. There must be produced enough of the "specific product" formed by the reaction of the tissues which are stimulated by the presence of the tubercle bacilli, in order to react with the tuberculin to produce the fever. Just how long a period of incubation is required is not known. It probably varies considerably in individual cases from a few weeks to several months.

An important series of tests described by Moore[6] may serve to illustrate the failure of the tuberculin test to detect all cases of tuberculosis in a herd at a single test. In January, 1904, a herd of 491 cattle was tested with tuberculin. Of these only 96 cows failed to react. The stables were disinfected with five per cent carbolic acid and the reacting animals all immediately removed. The apparently healthy animals were tested every six months afterward and all subsequently reacting animals promptly removed. The non-reacting animals were guarded carefully from reinfection. The result was as follows:

1904	Jan.	491	tested	395	reacted	96	did	not	react	80.4 percent reacted
1904	July	96	tested	31	reacted	65	did	not	react	32.2 percent reacted
1905	Jan.	65	tested	8	reacted	57	did	not	react	12.3 percent reacted
1905	July	57	tested	15	reacted	42	did	not	react	26.3 percent reacted
1906	Jan.	42	tested	15	reacted	27	did	not	react	35.7 percent reacted
1906	July	27	tested	3	reacted	24	did	not	react	11.1 percent reacted
1907	Jan.	24	tested	2	reacted	22	did	not	react	8.3 percent reacted
1907	July	22	tested	1	reacted	21	did	not	react	4.5 percent reacted
1908	Jan.	21	tested	1	reacted	20	did	not	react	4.7 percent reacted
1908	July	20	tested	1	reacted	19	did	not	react	5.0 percent reacted

Several of these animals which failed to react at the first test but did react later were examined *post mortem*. The lesions were slight in extent but of long standing and surrounded by a wall of fibrous tissue. Moore thinks these cows were infected and the lesions healed or partially healed before the first test January, 1904. Afterward the arrested lesion became active. So the oscillation of the morbid process between an active and an arrested condition is an important factor in the result of the tuberculin test.

The cow may fail to react during the early or incubation period, or at a time when the lesions are healing or partially healed but also *the reaction may fail in very advanced cases.* While this last is rare and in such cases the disease may usually be recognized by a physical examination, yet it may escape recognition, and such an occurrence is liable to cause very serious trouble. For such an animal is usually passing out enormous numbers of the tubercle bacilli from her body and may quickly infect a large part of the other animals in the herd. Special care should be exercised to detect these very important cases.

Recognition by Microscopic and Animal Tests

Special laboratory procedures are useful in detecting the dangerously tuberculous cow; to detect the cow that is constantly passing virulent tubercle bacilli in the milk, feces, or other secretions in order that she may be removed from the herd. Microscopic examination may be useful where a very bad udder is sus-

pected or something of this nature; but this test can not be relied upon unless the organisms are found in great numbers. Negative results are practically worthless. The testing of feces by microscopic examination and by animal inoculations has already been discussed. Uterine and nasal discharges may be tested by similar methods. In many European countries the testing of milk by guinea pig inoculations in order to discover the cow with tuberculous udder is a common laboratory practice. Mention of this subject has been made in connection with the report of Mueller's work. (See page 344.)

HARPOONING Another means of testing is by harpooning. This is more frequently used to determine the nature of knots or supposed tubercules in the udder, especially, or upon any part of the body. A harpoon, which is a needle with a beard at the tip, is thrust into the suspected swelling, revolved once, and quickly withdrawn. It brings with it, on the hook, a small bit of tissue which can be examined directly with the microscope or inoculated into animals. None of these methods can take the place of the tuberculin test but they are useful as supplements to this test.

DISPOSAL OF THE TUBERCULOUS COW

What shall be done with the tuberculous cow when she has been recognized? If the cow is wasting in body or physical signs of tuberculosis are evident, she should be killed at once and buried, or burned, or put in the rendering tank. When the cow is in apparently good or fair condition she may be valuable for beef, or she may be useful for the production of milk and the raising of calves for some years.

SLAUGHTER UNDER INSPECTION Slaughter under inspection is usually the most satisfactory means of disposal unless the cow is especially valuable for the production of milk and butter and the raising of calves. To the owners of small herds slaughter under inspection is to be recommended. The preservation in quarantine of the tuberculous portion of the herd according to the Bang method can sometimes be recommended to owners of large herds.

THE BANG METHOD.

What is known as the Bang method of handling a tuberculous herd in quarantine, and the building up of a healthy herd from a tuberculous herd was first carried out by Prof. Bernard Bang of the Royal Veterinary College of Copenhagen, Denmark. Bang's experience with the tuberculin test had shown him, that tho ac-

curately revealing the presence of tuberculosis it gave little knowledge of the extent of the disease in the cow, which might often indeed have only a slight infection. His special desire was to devise a system eventually to get rid of the tuberculous cow; to prevent at once further spread of the disease, and to interfere as little as possible with the breeding of cattle.

An outline of the method is as follows:

1. Apply the tuberculin test.
2. Separate the herd into reacting and non-reacting herds.
3. Provide for the two herds entirely separate stables, pastures, and attendants, if possible separate farms.
4. Thoroly disinfect the stables, watering troughs, and all utensils coming into contact with the healthy herd.
5. Test the well herd every six months, and remove the reactors and again thoroly disinfect the stables.
6. Take the calves of the tuberculous herd immediately from their mothers and feed them milk from non-reacting cows or milk pasteurized at 85° C.
7. Add no calves to the non-reacting herd except those showing a negative reaction to tuberculin, subcutaneously administered, at the age of six months.
8. No cows "bought in" must be added to the well herd until thoroly tested with tuberculin.
9. All milk from the tuberculous herd must be pasteurized or sterilized before used as food for man or animals.
10. As soon as an animal in the tuberculous herd arrives at an advanced stage of the disease indicated by physical signs, she should be immediately disposed of.

THE TUBERCULIN TEST IN THE BANG METHOD

The application of the tuberculin test should be carried out as previously described. This test should be made subcutaneously and should be interpreted with care. As has been said the positive results are quite reliable; but it is the negative results that give the trouble in applying the Bang method. Three things at least must be guarded as carefully as possible. 1. A cow that is very badly infected may fail to react. Most generally however her physical condition would at least show that the cow was not in a good state of health and should be discarded. One such cow may soon infect the whole herd and should be watched for with utmost care. 2. If the test was made during the period of incubation of a part of the cattle, which is almost sure to be the case in some of the animals, especially of a large herd in which this disease is just getting a start, some of the infected animals would not react; but later the disease would develop and

become evident in these. 3. In cases where the tubercles are sufficiently encysted and the disease becoming arrested, a reaction may not occur, as was shown by Moore (already cited). These cows may react at a subsequent test.

Because of these last two facts, the failure of the tuberculin to show the presence of virulent tubercle bacilli in the body of the cow during the period of incubation and also when the tubercle is sufficiently encapsulated to prevent the reaction, it is probably advisable when the larger part of the herd reacts, say seventy percent or more, not to separate it into two parts but to consider the whole herd tuberculous, and begin to build up a healthy herd from the non-reacting calves and thoroly tested cattle that are "bought in".

SEPARATION AND HANDLING OF THE TWO HERDS

After finding a smaller part of the herd tuberculous by the tuberculin test, the two herds should be immediately separated and provided with separate stables, feed lots and pastures. It is better when practicable to have separate farms. The two herds should be cared for by different persons. If this is not convenient, the hands should be provided with different sets of over-suits, and shoes for each barn so that no disease germs may be carried on their clothing from the tuberculous herd to the well herd. The healthy herd is tended first and then the diseased herd. One should never go directly from the tuberculous herd to the healthy herd.

DRINKING WATER

One of the serious difficulties is providing water for both herds. It is expensive to provide two sets of wells or cisterns, especially when in a few years one expects to be rid of the tuberculous herd and then will not need the extra well for water supply. The living germs may easily be carried in the water. Therefore in case there is only one source of water, it must be so arranged that the water will flow from the healthy herd to the tuberculous herd.

DISINFECTION OF THE STABLES

The stables where the non-tuberculous herd is to be kept must be thoroly disinfected. First, they should be thoroly cleaned, and then disinfected with mercuric chloride solution in proportion of one part of mercuric chloride to one thousand parts of water. The best way to apply this solution is with a spray pump. The wood work and floors of the whole stable, around the doors, the posts, fencing, and any other place on the premises where the tuberculous cows may have left tubercle bacilli, should be disinfected. After the disinfecting solution has dried, all the wood work should be painted, oiled, or white-washed.

RETESTING THE WELL HERD

It is necessary from time to time to retest the well herd to see that there remain no tuberculous animals. It is better to test every six months, and at least as often as once per year. The reactors must be immediately removed and the stables again thoroly disinfected each time. No cow should be tested for three weeks before or after calving, nor if she has an abnormal temperature from any cause; but if omitted from the regular test she should be tested as soon afterward as is convenient.

HANDLING OF THE CALVES

Calves of tuberculous cows very rarely contract tuberculosis from their mothers before birth. It is estimated that not more than four percent are tuberculous or become tuberculous at the age of six months if they are immediately removed from the mother at birth and fed wholesome milk. Even a calf from a cow with tuberculous genital organs may be born free from the germs. Every calf should be tested at the end of six months before it is permitted to enter the healthy herd.

COWS BOUGHT IN

Cows that are "bought in" must not be added to the well herd until they are thoroly tested. If they come from a region where there is considerable tuberculosis they should be kept from the non-tuberculous herd for 4 to 6 months until at least two tests have been made before putting them with the well herd. It is by far better to buy cows only from a herd known to be entirely free from tuberculosis. The non-tuberculous herd is the great desideratum when you are buying—much more to be sought than the non-tuberculous individual; because it is not unlikely that the non-tuberculous individual, proved so only by the tuberculin test, especially if she comes from a tuberculous herd or a region where tuberculosis is plentiful, may on the second or third test prove to be tuberculous; and the buyer may find it out only too late, to save his well herd from becoming infected.

PASTEURIZATION OF MILK BEFORE FEEDING TO HOGS AND CALVES

It is necessary to pasteurize all milk from the tuberculous herd before use as food for man or for animals. The best disposition of milk from tuberculous cattle is to use it in the manufacture of some of the dairy products, as butter, or evaporated cream. In these the thoro pasteurization does not harm the finished products. It is the lack of attention to pasteurization that has caused so much trouble in many of the dairy regions. A cow with a tuberculous udder can infect all the milk from a number of herds when this is mixed together in the large containers at a creamery, and the skim milk from this cream-

ery when fed to calves and pigs will produce tuberculosis. This is one of the most common causes of infection among these animals.

THUREBYLILLE FARM EXPERIMENT

Bang[1] applied his method first on the Thurebylille farm in 1892. A special government grant gave him the funds. When this herd was first tested 131 animals reacted and only 77, mostly young animals, were found to be healthy. The reactors and the non-reactors were at once separated but only with a board partition between them in the stables, the shed occupied by the calves forming part of the healthy division, which should have been separate. They were attended by two sets of hands. The cattle were kept entirely separate at all times both in the stable and pasture. At times the project was discouraging. The reactors at one time from the well herd were as many as 9 percent. Things necessary to be done were not clearly understood by the farmer and at times a highly tuberculous cow would be allowed to remain on the farm for some time, discharging immense numbers of virulent bacilli. In spite of all these drawbacks, year by year the healthy herd increased and in 1907, five years after the experiment started, the herd consisted of 211 animals without one reacting to the tuberculin test. This herd is now supplying "milk for infants" in the city of Copenhagen, a very superior milk. This experiment has been repeated so many times in Denmark that it has now come to be known as the Bang system. It is especially successful on the larger farms and estates, more particularly where the herds can be placed on separate farms. On such farms the herds have been cleared of tuberculosis in two or three years. But not only on the large farms but on the small farms this method of Bang has been successful. Bang[1] records in 1905 that on 66 small farms, with an average of 29 head of cattle per farm, there were 1045 reacting animals and 780 healthy animals. This method was put into practice and a few years later there were 1896 healthy animals and none reacting.

Harding and Smith[4] in New York State were equally successful in converting a tuberculous herd into a healthy herd. In Wisconsin[8] and in Pennsylvania[5] the Bang method has been applied with success. However both in the United States and in Canada the Bang method has not proved satisfactory to most of the stock owners who have tried it.

Klein[5], State Veterinarian of Pennsylvania, says that the Bang method is not popular and has not been adopted in his state except in a few instances. The reasons appear to be the restricted market for the heated milk and the extra labor in maintaining the two separate herds. Pope[7] of East Orange, New Jersey, quotes from a letter received from Hon. W. C. Edwards, Rockland, Ontario.

"I most certainly think that the Bang system is a most excellent one for any one to practice. I may say, however, that I do not think that many farmers or even breeders, will go to the expense and trouble involved in the practice. Personally I may say that the riddance of tuberculosis thru the Bang system is very effective; but it is very expensive to practice it, as we practice it here. If it was to do over again I would have separate farms....."

"ASSOCIATE FARM"

It seems plausible that an "associate farm" where the neighbors could combine their forces in stamping out tuberculosis from among their cattle would be a good thing. There the owners of small herds could economically use the Bang method in ridding their herds of this disease.

It would only be necessary to have a small tract of land sufficient to pasture the tuberculous cows kept by the association. The tract of land, with sufficient buildings could be owned, rented, or leased as each association should choose for itself. The "associate farm" would take care of the tuberculous cow or cows for each member of the association for which would be paid a stipulated price agreed upon by the members. Only cows valuable either for butter making or more especially for their calves would be expected to be kept on the farm. Each member would get back his own calves to add to his sound herd upon his own farm, and have also his share of the proceeds of the dairy products that were marketed.

The advantages of the "associate farm" would be several: 1. The disease would be much more completely isolated. The tuberculous cows of the whole neighborhood could easily be kept from the non-tuberculous cows. The method would thus avoid in a large measure the difficulties and expenses of the Bang system as practiced by the individual farmer, and moreover would insure greater protection for the healthy herds. 2. There would be an advantage in marketing the dairy products. The public interest in the matter of pure dairy products is becoming more and more pronounced, and when any dairyman could show that he had no tuberculous cattle upon his farm, his dairy products would be at a premium. The cream from the tuberculous herd at the association farm could be made into the highest grade butter after proper pasteurization. The method thus combines the best principles of the Bang system and does away with the expense of two sets of stables and pastures and two sets of hands at each farm. It provides the small stock owner the opportunity of successfully using the Bang method to rid his herd of tuberculosis, and at the same time gives him an advantage in marketing his dairy products.

THE DUTY OF THE STOCK OWNER

There is expense involved, and every farmer will have to determine for himself whether the expense in freeing his herd from tuberculosis by the Bang method will be greater than that of slaughtering and purchasing new stock. There will be expense, trouble and aggravation in any way he rids his farm of this dreaded disease. But the expense will not be so great as keeping the disease in his herd, which sooner or later is sure entirely to destroy it. The problem is here and it must be faced, and the sooner the fight is begun, for a fight it must be, the less will be the struggle and expense. Moreover, in the end, this will be the task of the individual farmer in whose herd the disease exists. Indeed the Nation and the State will have and should have their share of responsibility. But tuberculosis like a noxious weed must be kept out of each individual herd, and the cattle owner is the only one that can constantly keep guard over his own flock. To this end the stock owner should educate himself concerning tuberculosis, concerning its slow development in the animal body, the ease with which it is overlooked, its contagiousness, its manner of spread from herd to herd and from animal to animal within the herd, its detection by the tuberculin test and by other means; and he should learn to understand especially the failure that he may experience by relying too implicitly upon the negative tuberculin test. More than this, not only must he inform himself upon this subject, but he must help teach his neighbor; for his ignorant neighbor may maintain a tuberculous herd in such condition as to menace the stock of all the surrounding farms.

DUTY OF THE STATE

It is widely recognized that it is an obligation of the state to take an active part in the control and prevention of tuberculosis among farm animals. For this purpose Germany and England have appropriated large sums of money to carry on scientific investigations which they are doing thru their Commissions on Tuberculosis. A large amount of investigation has been done in this country by the United States Government thru its Bureau of Animal Industry, as well as by various state governments. As has been said before, in the end it will fall to the lot of the stock owner to eradicate and to keep out of his own herd this dreaded disease. But it will be the part of the state to organize the work, to combine the efforts of the various live stock interests, health organizations and other societies that they may all work together in harmony to one end, the complete eradication, finally, of tuberculosis from among the farm animals. This is possible. An example of effective prevention may be seen in the Island of Jersey where by vigilant care the cattle are kept quite free from tuberculosis.

ORGANIZATION OF THE WORK

The tuberculosis work of the state is usually organized and carried out under a Board of Live Stock Commissioners. This is better than the creation of a special board for this single disease. Extra funds will be necessary and should be provided so this board may secure the proper help to carry on its work efficiently. It would be folly for this board to attempt a radical elimination of all tuberculous cattle. This has been tried in other states and found impracticable. It will be necessary to proceed along conservative lines. It is the combined effort of all concerned that is the most effective in eliminating this disease. To preserve the harmony and to keep at work all the organizations concerned in eradicating tuberculosis from farm animals will be one of the most difficult as well as the most important of the tasks that the Board of Live Stock Commissioners will have to perform.

FREE TUBERCULIN TESTING

Another important work of the Board should be the providing of free tuberculin testing of cattle when requested by stock owners. This testing should be done under proper restrictions and control. The stock owner should submit to such a control of the reacting animals that they may not become a menace to other stock and to the health of the people. Each reacting cow should be branded by cutting the letter T in the right ear, so that the few unscrupulous stock owners could not impose upon their neighbors by selling the fat, sleek, reacting cows for healthy animals. This testing should be free, for then it will be done more usually by authorized experts, which is necessary for the best results. Furthermore the report of the testing which is so essential for the further and effective control of the reacting cattle will thus be in the hands of the state officials.

CONTROL OF THE REACTING ANIMALS

All reacting animals that are not especially desired for breeding purposes or for the production of dairy products should be slaughtered under State or Federal inspection. When it is desired to retain such reacting animals as mentioned above, proper quarantine regulations should be enforced under the control of the Board of Live Stock Commissioners. Printed regulations and advice should be provided by this board when requested by the owners of such stock. An inspector should visit the quarantined herd not only to see that the regulations are carried out but to help the stock owner by advising him of the best and most scientific methods of ridding his herd of tuberculosis. The sale of tuberculous animals *except as such* should be prohibited by law.

An indemnity of the real value of a tuberculous cow should be

paid the owner when it is the opinion of a state official that a cow should be destroyed in order to protect the stock of the surrounding neighborhood. This procedure should be used sparingly and only when absolutely necessary. It should be remembered that very few states, if any, have ever succeeded in ridding themselves of tuberculosis by buying up the tuberculous animals. Maine may possibly prove to be the one exception, and Maine can succeed, if she does finally, only because there is very little tuberculosis in the state. Illinois can not wisely undertake such a procedure at present. It is especially unwise to pay a *fictitious price* for such indemnity. The value of a tuberculous cow can never equal the price of a non-tuberculous cow of similar grade however well she may look from outward appearances. The indemnity should be measured by the actual market value of the tuberculous cow recognized as tuberculous.

PUBLIC LISTING OF NON-TUBERCULOUS HERDS

To encourage stock owners to keep their herds free from tuberculosis it seems advisable to keep and publish lists of owners of such herds, indicating the size of each herd, and the time it has been kept free from this disease as shown by official testings. Such publications may be undertaken by the various live stock associations but the testing and reporting should be done under the direction and control of the Board of Live Stock Commissioners. The records of the Board should be available to any purchaser and not only would it help the purchaser to select stock from herds free from tuberculosis as shown by official tests, but it would be an incentive to stock owners to keep their herds free from this disease as it would put a premium upon such herds.

EDUCATION POPULAR AND SPECIAL

Another important duty of the state is to carry on popular education along these lines. This should be promoted and carried out by all organizations concerned and at all available opportunities. There is not any one thing that will do more to rid farm animals of tuberculosis than the education concerning this disease of every person who has to deal with such animals. Public demonstrations showing the value of the tuberculin test by slaughtering some of the reacting animals, especially some of those whose physical appearance is that of healthy cattle, with the demonstration of the lesions in such cattle, will convince the most skeptical of the worth of this valuable test. Popular lectures and exhibitions of the tuberculous tissues from cattle and hogs will be very profitable.

It might also prove economical and wise for the state to provide facilities for the training of men expert and skillful in the handling of this disease. At least good general instruction in the subject for all agricultural college students would seem advisable.

SUMMARY

1. Recognition of tuberculosis in dairy cattle depends almost entirely upon the tuberculin test.

2. The positive tuberculin test is accurate in about 98 percent of the cases as shown by slaughter; the negative test is not so reliable.

3. For handling the tuberculous cow one of the following methods is advised:

a. In advanced cases where there are physical signs of the disease or when the cow is not very desirable for breeding or dairy purposes, slaughter under inspection is best.

b. When it is desirable to keep the reacting cow for the purposes mentioned above, the Bang method is recommended especially to large stock owners.

c. For owners of smaller herds the modified Bang method by means of a stock owners' association, so the tuberculous animals may be farther removed from the healthy herd, is to be recommended.

4. The responsibility of ridding farm animals of tuberculosis must be placed upon the stock owner before there can be any hope of permanent success; for only the stock owner can keep close watch over his herd.

5. The State will have her share of responsibility in formulating rules and regulations, providing for free tuberculin testing, and carrying out a system of popular and special education upon the subject of animal tuberculosis.

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